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Abstract Book

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1 **Larry Sandler Award Lecture - How proteolytic degradation, actomyosin contractility, and cell polarity converge to regulate Hippo signaling and tissue growth** Sherzod A Tokamov Plant and Microbial Biology, The University of Chicago

The Hippo pathway is an evolutionarily conserved regulator of tissue growth. At the core of the pathway, a kinase cascade represses the activity of a transcriptional effector, an oncoprotein called Yorkie. Inactivation of the Hippo pathway results in the translocation of Yorkie into the nucleus, where it promotes a pro-growth genetic program. Multiple upstream inputs are known to synergistically activate the kinase cascade from the apical cortex of polarized epithelial cells. However, how these components are organized and the mechanisms by which they are regulated remains poorly understood.

My thesis work explores how Hippo signaling is controlled, focusing on the regulation and organization of a key upstream Hippo pathway organizer, a multivalent scaffold protein called Kibra. Unlike other Hippo pathway regulators, which localize mainly at the junctional cortex of epithelial cells, Kibra's subcellular localization is distinctly partitioned into junctional and medial domains of the apical cortex. Previous work has shown that medial localization potentiates Kibra-mediated Hippo pathway activation, but the mechanisms that control Kibra activity and subcellular organization remain unknown.

In this dissertation, I present evidence that proteolytic degradation, actomyosin cytoskeleton, and apical polarity network converge to control Kibra-mediated Hippo signaling. First, I demonstrate that ubiquitin-mediated degradation is a major mechanism that regulates Kibra abundance. Specifically, upon assembly of the Hippo complex, Kibra is ubiquitinated via the SCF^{Slimb} E3 ubiquitin ligase machinery and is subsequently degraded. Next, I show that ubiquitin-mediated Kibra turnover is modulated by actomyosin-generated cortical tension. Mechanistically, increased tension results in tighter cortical association of the Ser/Thr kinase Par-1, and Par-1 promotes Kibra degradation. Finally, I identify the mechanism by which Kirba is partitioned into junctional and medial pools at the apical cortex. I show that the apical polarity network, in part via aPKC, tethers Kibra at the junctional cortex to silence Kibra-mediated Hippo signaling, whereas medial actomyosin flows untether Kibra from the junctional cortex and promote its medial accumulation, thereby increasing Kibra-mediated Hippo signaling. Together, these findings provide crucial insights into the regulation of the Hippo pathway and reveal functional relationships between upstream Hippo signaling, actomyosin dynamics, and apical polarity network in tissue growth control. More broadly, this work provides a paradigm for understanding how mechanical forces and epithelial cell architecture organize and regulate intracellular signaling events.

2 **Defective *Hoxb8* Microglia are Causative for both Chronic Anxiety and OCSD-like Behavior in Mice** Mario Capecchi Human Genetics, Univ Utah Sch Med

We have shown there are two cell lineages in mice that give rise to microglia with distinct ontogenies; *Hoxb8* microglia and non-*Hoxb8* microglia. Disruption of *Hoxb8* or ablation of the *Hoxb8* cell lineage gives rise to both chronic anxiety and OCSD-like behavior (trichotillomania, compulsive pathological overgrooming, leading to lesions at the sites of overgrooming). There is a strong sex bias for both behavioral pathologies, with females showing much more aggressive disease. By cell transplantation of purified, cell sorted *Hoxb8* microglia progenitors derived from Wt or *Hoxb8* mutant mice we were able to demonstrate causation of defective *Hoxb8* microglia for both behavioral pathologies. Recently we have demonstrated that optogenetic activation of *Hoxb8* microglia in specific regions of the brain induces higher levels of anxiety, grooming or both. Further, these experiments have demonstrated that *Hoxb8* microglia function to reduce anxiety and grooming (function as brakes) whereas non-*Hoxb8* microglia function to increase these behaviors (function as an accelerator). This provides a biological reason for mice having two populations of microglia. We have further extended this work to define the molecular mechanisms used by microglia to communicate with specific neuronal circuits to modulate anxiety and grooming in mice.

3 ***Doublesex* modifies wing circuits in the evolution of courtship song types in *Drosophila*** Dajia Ye, Justin Walsh, Ian Junker, Yun Ding Department of Biology, University of Pennsylvania

Animals exhibit extraordinary behavioral diversity. However, it is unclear how species variation in complex motor behaviors are encoded by evolutionary changes in genes and neurons. To fill in this knowledge gap, we took advantage of the diversification of *Drosophila* courtship songs as a model system to explore the molecular and neural mechanisms underlying species variation in behavior. Males of *Drosophila* species sing species-specific and context-dependent courtship songs by wing vibration. In many *Drosophila* species including *D. melanogaster*, males sing two major types of songs, sine song and pulse song. In contrast, *Drosophila yakuba* has lost the ability to sing sine song. Here we investigated the lineage-specific loss of sine song in *D. yakuba* by comparing its neural circuit with *D. melanogaster*. We found that the loss of sine song is accompanied by a partial loss of TN1 neurons – a group of male-specific wing patterning neurons. Anatomical and single-cell transcriptomic comparisons of TN1 neurons between species support a loss of the TN1A neuronal subtype, which is essential for sine song generation in *D. melanogaster*. Consistent with this cell-type specific loss, optogenetic activation of TN1 neurons in *D. yakuba* triggered the characteristic unilateral wing extension but not sine song. To explore the genetic underpinning

of TN1 neuron divergence, we leveraged the involvement of the sex determination gene *doublesex* (*dsx*) in TN1 neuronal development in *D. melanogaster* males to test if the function of *dsx* evolved to cause the partial loss of TN1 neurons in *D. yakuba*. Whereas *dsx* expression had no effect on the number of TN1 neurons in *D. melanogaster*, we found that in *D. yakuba*, loss of *dsx* led to more TN1 neurons compared to control males. Surprisingly, blocking apoptosis of *dsx* neurons also resulted in a gain of TN1 neurons to the same extent as *dsx* null males, which indicates that *dsx* evolved to promote TN1 apoptosis in *D. yakuba*. Together, our work revealed a causal role of cellular changes in motor patterning circuits during the evolution of complex behaviors, and the evolutionary lability of sex determination genes in reconfiguring sexually dimorphic circuits through programed cell death.

4 **Rock (dove) 'n' roll: the genetic basis of rolling in the domestic pigeon (*Columba livia*)** Atoosa M Samani, Tori M Earl, Emily T Maclary, Michael D Shapiro University of Utah

Hereditary rolling in the domestic pigeon (*Columba livia*) is an unusual behavior characterized by backward somersaults when attempting to fly. Rolling is progressive: it does not present itself until a few weeks after fledging and becomes more severe with age. Roller pigeons walk, eat, and breed normally, suggesting that rolling is a specific context-dependent behavior and not a generalized physiological disorder. Rolling is recessive and highly heritable – Charles Darwin described it as "…one of the most remarkable inherited habits or instincts ever recorded" – yet the molecular genetic basis of this behavior remains unknown. Therefore, rolling offers a unique opportunity to discover the molecular basis of a complex yet genetically tractable behavior. In a laboratory intercross between rolling and non-rolling pigeon breeds, we identified several quantitative trait loci (QTL) associated with rolling behavior, with one major QTL explaining 56% of the phenotypic variance. Comparisons between the re-sequenced genomes of 25 roller and 125 non-roller pigeons confirmed the polygenic control of this behavior, and our complementary QTL mapping and GWAS approaches yielded overlapping results. We also compared the transcriptomes of different brain regions of roller and non-roller pigeons and found that the cerebellum has the highest number of differentially expressed genes. Pathway analysis suggests that translation is inhibited, and synaptogenesis is activated in the hindbrain of rollers, similar to neurodegenerative diseases. Dissection of the candidate loci at the gene level will deepen our understanding of the molecular basis of involuntary and task-specific movement disorders and other progressive vertebrate behaviors.

5 **IGEG-1 is an EGFR ligand that links cellular stress with sleep in *C. elegans*** Cheryl Van Buskirk[1], Andrew Hill[2], Bryan Robinson[3], Paul Sternberg[2] [1]Biology, California State University Northridge, [2]California Institute of Technology, [3]California State University Northridge

Across animals, prolonged wakefulness is associated with indicators of cell stress in both neuronal and non-neuronal tissues, indicating that a core function of sleep is to promote global cellular homeostasis. Further, in both invertebrates and vertebrates, increased sleep is observed in response to conditions that cause cell damage, DNA damage, or disruption of protein homeostasis. The mechanisms by which cellular stress promotes sleep, and by which sleep in turn restores homeostasis, are poorly understood. The nematode *C. elegans* provides a compelling system for addressing these questions, as exposure to damaging conditions triggers sleep rapidly, independent of circadian cues. This stress-induced sleep (SIS) can be modulated by mutations that impact cellular repair processes, and appears to aid recovery from noxious exposure. Neurons that mediate SIS have been identified and shown to depend on EGFR/LET-23 signaling for their sleep-promoting function. However, the putative EGF signal and the mechanism by which it transduces diverse cellular stressors are unknown. We have recently identified IGEG-1, a previously unrecognized *C. elegans* EGFR ligand that is critically required for SIS. We present evidence that the extracellular EGF domain of IGEG-1 undergoes stress-responsive shedding by the ADAM17/TACE homolog ADM-4. We find that inducible overexpression of IGEG-1 potently induces sleep that depends on ADM-4 and LET-23, and that the requirement for ADM-4 can be bypassed using a constitutively-secreted form of IGEG-1. We have employed the cGAL-UAS system to identify the site of IGEG-1 release during SIS and we find that the site of sheddase activity depends on the tissue specificity of the damaging agent. Our data support a model in which IGEG-1/EGF is released from damaged tissues to promote sleep by activating EGF receptors in sleep-promoting neurons. Our study identifies a molecular mechanism linking the accrual of cellular stress in diverse tissues with organismal sleep drive, with potential bearing on our understanding of sleep in humans.

6 **Evolutionary genetics of aggression of the Siamese fighting fish** Pei-Yin Shih, Hiroki Tomida, Alec Palmiotti, Claire P Everett, Marcos Valenzuela Cuevas, Sarah Aktari, Andrés Bendesky Columbia University

Aggression is a fundamental social behavior that animals use for gaining and defending resources, territories, and mates. Genetic variation is known to contribute to individual differences in aggression, but the specific genes involved are still largely unknown. To identify these genes and how their variation mechanistically shapes aggressive behavior, we are leveraging the unique traits of the Siamese fighting fish, *Betta splendens* (betta). Highly aggressive fighting betta have been generated through more than 700 generations (~400 years) of artificial breeding. Their aggressive behaviors are easily triggered by visual stimuli, and their intense fighting continues until retreat or death. To characterize their behaviors, we used highly controlled

and reproducible visual stimuli, including custom fish animations and 3D-printed fish models, and found that fighting betta show more aggressive displays as well as physical biting attacks compared to their unselected wild counterpart. To identify genetic variants under selection in fighting betta, we used population genetic approaches and identified a strong genetic signal of selection in fighting fish peaking at the *neuroligin1* (*nlgn1*) gene. *nlgn1* mediates the formation and maintenance of synapses and its human homologs have been implicated in the etiology of autism spectrum disorder, which often includes high aggression. There were no coding differences in *nlgn1* between fighting and wild fish, but fighting fish express 2–4×-less *nlgn1* mRNA during larval development than wild fish. We are now performing genetic manipulations to confirm the involvement of *nlgn1* and further characterizing its mechanism of action. Notably, we have developed multiple genetic tools in betta fish, including CRISPR/Cas9-mediated knockout, CRISPR/Cas9-mediated knock-in and Tol2-mediated transgenic methods. This study is providing insights into molecular, genetic, and neuronal mechanisms of aggressive behavior, questions of fundamental importance in the study of the evolution and biology of animal behavior.

7 **Cellular and molecular basis of an aversive pathway regulating drinking in flies** Anindya Ganguly[1], Craig Montell[2] [1]Neuroscience Research Institute, University of California, Santa Barbara, [2]Neuroscience Research Institute; Molecular, Cellular and Developmental Biology, University of California, Santa Barbara

Drinking or consumption of water plays a pivotal role in regulating the osmolality of bodily fluids, thus contributing significantly to the maintenance of osmotic balance in the body. In fruit flies, detection of water by their taste system precedes initiation of drinking bouts. Previous studies have identified that *ppk28* an amiloride-sensitive channel functions as the water receptor in specific sub-class of gustatory sensory neurons dedicated to water perception. Knocking out *ppk28* or artificially silencing these neurons substantially reduces drinking time in flies. We report an additional pathway that perceives water as having a negative valence, influencing the duration of drinking and thereby contributing to the regulation of water intake. We observed that *OtopLA*, a proton channel previously been shown to function as a broad-range pH sensor in flies, can also function towards the detection of water. However, contrary to what was previously observed with *ppk28*, drinking time was prolonged by 4-5 times in flies where *OtopLA* was knocked out. By conducting cell-type specific genetic rescue experiments in various subsets of taste neurons, we demonstrated that *OtopLA* function is required in a subset of aversive taste neurons previously recognized for their sensitivity to high salt concentrations. Silencing these neurons significantly extended drinking duration, phenocopying OtopLA mutant flies. We propose a model in which the relative frequencies of action potentials between two subsets of neurons, one detecting water as a positive stimulus and the other perceiving it as a negative one, jointly regulate drinking behavior in flies. Specifically, a higher frequency of action potentials in *ppk28* water taste neurons signals the initiation of drinking, while the *OtopLA* water neurons convey signals to terminate drinking episodes. As the function of otopetrins as proton channels is remarkably conserved, this study lays a solid foundation for investigating drinking regulation in various other animal species.

8 **Caregiving Drives Changes in Male Behavior, Neural Physiology, and Gene Expression in California Mice** Maria E Colt[1], Erica Glasper[2], Heidi Fisher[3] [1]Biology, University of Maryland, [2]The Ohio State University College of Medicine, [3]The Jackson Laboratory

Parental care is critical to offspring survival in mammals but can also lead to measurable changes in parents. In the biparental California mouse, *Peromyscus californicus*, fathers actively groom, huddle, retrieve, and build a nest for their offspring. Previous research has shown that these fathers experience increased memory, decreased anxiety, hippocampal neurogenesis, and increased density of dendritic spines, which improves contact between neurons. However, the trigger, reproduction or caregiving behavior, and molecular pathways that regulate these behavioral and neurophysiologic changes, remain unclear. Here we first compared caregiving behaviors in fathers who have cared for their pups and pup-sensitized non-fathers who have cared for unrelated pups and found that pup-sensitized non-fathers were slower to approach pups but eventually spent more time grooming pups, whereas fathers spent more time nest-building. We also compared recognition memory and anxiety-like behaviors, as well as hippocampal dendritic spine density in fathers, non-fathers with no caregiving experience, pup-sensitized non-fathers, and virgins that were paired with another male and had no caregiving experience. We found that experienced fathers exhibited increased recognition memory and decreased anxiety-like behavior compared to virgins and non-fathers, and that fathers show the greatest dendritic spine density. Finally, we compared hippocampal gene expression in fathers, non-fathers, and pup-sensitized non-fathers and performed network and pathway analyses to identify suites of RNA expression patterns associated with caregiving experience. We found that fathers exhibit an upregulation of genes associated with neuron generation, differentiation, and development, but that pup-sensitized non-fathers also exhibit many of these same gene expression changes. Lastly, we found that caregiving experience is associated with specific biological pathways and newly identified expression modules. Our results suggest that caregiving behavior induces important behavioral, structural, and molecular changes in the brains of males even if the offspring are not their own.

9 **Transgenic worms for interrogating signal propagations in *Caenorhabditis elegans* using GUR-3+PRDX-2 as neuro-actuator** Anuj Sharma[1], Francesco Randi[1], Sandeep Kumar[2], Sophie Dvali[1], Andrew Leifer[3] [1]Department of Physics, Princeton

University, [2]Princeton Neuroscience Institute, Princeton University, [3]Department of Physics and Princeton Neuroscience Institute, Princeton University

The *Caenorhabditis elegans* nervous system has the most mature and comprehensively mapped connectome, or wiring diagram of chemical and electrical synapses of any animal (White, Southgate et al. 1986, Cook, Jarrell et al. 2019, Witvliet, Mulcahy et al. 2021). Although the connectome has been an invaluable resource, it does lack some information about the strength and sign (excitatory vs inhibitory) of neural connections or their temporal properties and cannot capture extrasynaptic signaling of mono-amines and neuropeptides (Bentley, Branicky et al. 2016, Ripoll-Sánchez, Watteyne et al. 2022). Instead functional recordings of neural signaling in living animals, including nematodes (Guo, Hart et al. 2009), can in principle capture these other properties of the nervous system by activating individual neurons and measuring neural activity in response, what we call signal propagation. We sought to achieve signal propagation recordings at whole brain scale. So, we created a suite of transgenic lines to optically measure direct signal propagation between neuron pairs, at brain scale and cellular resolution in the nervous system of the nematode *C. elegans*. Using these strains, we have found that peptidergic signaling also contributes to neural dynamics in the animal's head (Randi, Sharma et al. 2023).

To achieve this, we generated transgenic animals that express the calcium indicator GCaMP6s in nucleus of each neuron and a purple-light-sensitive actuator, the gustatory receptor homolog system GUR-3+PRDX-2 a peroxiredoxin, in each neuron (Bhatla and Horvitz 2015). This combination of indicator and activator allows for 2-photon targeted optogenetic stimulation of GUR-3 while simultaneous 1-photon calcium imaging of GCaMP6s with minimal optical cross-talk. To achieve high expression levels for avoiding neuro-actuator related toxicity, we used the QF-GR drug-inducible system to turn on gene expression only in adulthood (Monsalve, Yamamoto et al. 2019). These optogenetic tools were expressed in a NeuroPAL background to allow each neuron to be uniquely identified (Yemini, Lin et al. 2021). We named it TWISP**,** a Transgenic Worm for Interrogating Signal Propagation, because it enables optical measurements of evoked calcium activity at brain scale and cellular resolution in the nervous system of *C. elegans* (Randi, Sharma et al. 2023, Sharma, Randi et al. 2023).

In addition, new lines with *unc-13 (s69)* & unc-13 *(e51)* as well as *unc-9 (fc16);unc-7(e5)* mutant back grounds by out crossing and *inx-1(wtf511)* & *inx-7 (wtf514)* by CRISPR Cas9 KO are created for further investigations into neurotransmitter and gap-junction mediated signaling. These transgenic worms will be a resource for large scale investigations of functional connectivity in the brain.

10 **Understanding and exploiting murine tolerance of haploinsufficiency in human disease research** Kyle Drover[1], Amrit Puarr[1], Neil Horner[2], Zoe Thomas[1], Kristen Barratt[1], Henrik Westerberg[2], Ruth Arkell[1] [1]The John Curtin School of Medical Research, The Australian National University, [2]Medical Research Council Harwell Institute

In many murine models of human dominant diseases, the analogous phenotype shows recessive inheritance, a feature known as murine tolerance of haploinsufficiency. In fact, analysis of mouse phenotype databases revealed that, of 1346 human autosomal dominant diseases with a corresponding mouse model, 715 (~53%) of the mouse models exhibit murine tolerance of haploinsufficiency such that the disease phenotype is inherited in a recessive manner. We have investigated this phenomenon using the kumba (*Ku*) mouse model of *ZIC2*-associated holoprosencephaly (HPE). In humans this devastating congenital defect, in which the brain fails to split into the left and right hemispheres, is overwhelmingly associated with heterozygous deleterious mutations, with most solved cases associated with loss-of-function mutations in the *ZIC2* or *SHH* gene. Several mouse alleles of *Zic2* exist and all lead to HPE only when the mutation is homozygous. We hypothesised that a combination of differences in the way in which human and mouse populations are studied could account for the altered inheritance pattern. By studying larger populations on a range of genetic backgrounds and using advanced phenotyping tools like those used in humans we found the *Zic2^{Ku}* model exhibits a dominant, incompletely penetrant HPE phenotype on a certain genetic background. This closely mirrors the mode of HPE inheritance in humans and demonstrates that murine tolerance of haploinsufficiency may be an artefact of the way disease models are typically analysed. Furthermore, by using automated analysis of 3D images of embryos heterozygous for the *Zic2^{Ku}* mutation, we identified a HPE signature, facilitating AI-based diagnosis of HPE. We are now combining AI diagnosis with knowledge of HPE permissive and restrictive genomes to identify genes that enhance or suppress the *Zic2*-associated HPE phenotype. Rather than murine tolerance of haploinsufficiency being a drawback of mouse models, it can be exploited to identify alleles of small effect that contribute to clinical heterogeneity, the source of which is difficult to find using human studies alone.

11 **Zebrafish pre-clinical models link oxidative stress with intervertebral ECM defects and identify elevated spine stiffness as a prognostic biomarker and possible therapeutic target for adolescent idiopathic scoliosis** Josh Gopaul[1], Patrick Pumputis[2,3], Ran Xu[2,3], Jenica Van Gennip[2,3], Nikan Fakhari[1,4], Jerome Baranger[1], Olivier Villemain[1,4], Brian Ciruna[2,3] [1]Translational Medicine Program, The Hospital for Sick Children, [2]Developmental & Stem Cell Biology Program, The Hospital for Sick Children, [3]Department of Molecular Genetics, University of Toronto, [4]Department of Medical Biophysics, University of Toronto

Adolescent idiopathic scoliosis (AIS) is a pediatric disorder characterized by rotational spinal deformity, which develops in the absence of obvious congenital or physiological defects. 4% of children will develop AIS, and 1 in 10 patients (predominantly female) will experience severe curve progression. Patient exome/genome sequencing and mouse functional studies have associated genetic variants in musculoskeletal collagen and cartilaginous extracellular matrix (ECM) defects with a fraction of AIS cases. However, GWAS meta-analyses estimate that >95% of total genetic variance underlying AIS remains to be discovered. To date, the biology of AIS remains poorly understood, there are no prognostic biomarkers, and treatment options are limited to restrictive bracing and corrective surgery.

Using zebrafish models of AIS, we have discovered that *oxidative stress* and *pro-inflammatory signals* in the spinal cord, which develop because of cerebrospinal fluid homeostasis defects, are necessary and sufficient to drive spine curvature. Indeed, antioxidant and immunomodulating drugs can efficiently block scoliosis onset and severe curve progression in fish models. Although this provides proof-of-principle that AIS might be managed therapeutically, uncertainties regarding downstream mechanism and their link to human disease pose a barrier to clinical translation.

Here, we demonstrate that oxidative stress in fish AIS models induces an unfolded protein response that is associated with collagenous ECM defects within developing intervertebral spine segments. Using shear wave elastography (SWE), we show that zebrafish scoliotic spines are consequently stiffer than healthy controls - a property also reported for intervertebral discs in human AIS patients. Remarkably, zebrafish are significantly stiffer *prior* to scoliosis onset and increasing axial stiffness positively correlates with curve severity, suggesting a causal role for elevated stiffness in AIS. Finally, we demonstrate that antioxidant drugs known to suppress scoliosis may ultimately function by reducing spine stiffness to normal levels, providing a possible mechanistic link between oxidative stress and connective tissue/intervertebral defects identified in both fish and human AIS studies. As SWE is non-invasive and widely applied in the clinic, tissue stiffness may translate into a valuable prognostic biomarker and therapeutic target for AIS.

12 **Interneuron migration is controlled by *Dyrk1a* dosage via deregulations of actomyosin dynamics during corticogenesis, insights for the *Dyrk1a* haploinsufficiency syndrome pathogenesis** Yann Herault, Maria Victoria HINCKELMANN, Aline Dubos, Victorine Artot, Gabrielle Rudolf, Thu Lan Nguyen, Peggy Tilly, Valerie Nalesso, Maria del Mar Muniz Moreno, Marie-Christine Birling, Juliette Godin, Veronique Brault Institute of Genetics and Molecular and Cellular Biology (IGBMC)

Interneuron development is a crucial step during cortical morphogenesis of the brain. DYRK1A is a dual-specificity tyrosine phosphorylation-regulated kinase whose dosage is crucial for the development and the functioning of the brain. When present in only one copy in the mental retardation autosomal dominant 7, *DYRK1A* induces a syndromic form of intellectual disability, with autism spectrum disorder and epilepsy as common comorbidities. Mutations on *DYRK1A* are the underlying genetic cause of the disease, leading to cortical excitatory/inhibitory imbalance. However, how this imbalance is established remains elusive. Here, using mouse models and live imaging, we show that *Dyrk1a* specifically regulates the development of the cortical GABAergic system. Unlike projection excitatory neurons, we demonstrate that interneuron tangential migration relies on *Dyrk1a* dosage and kinase activity through a mechanism involving actomyosin cytoskeleton remodeling. Interestingly, we further demonstrate that mice with heterozygous inactivation of *Dyrk1a* in interneurons show behavioural defects and epileptic activity, recapitulating phenotypes observed in human patients. Altogether, these data highlight the critical role of *Dyrk1a* in the development of the GABAergic system and the pathophysiology of *DYRK1A*-haploinsufficiency syndrome.

13 **A *C. elegans* Model of Fanconi Anemia Neurological Syndrome (FANS)** Jessica E Leighton[1], Belinda Barbagallo[2], Niall G Howlett[1] [1]Cell and Molecular Biology, University of Rhode Island, [2]Biology and Biomedical Sciences, Salve Regina University

Fanconi Anemia (FA) is a rare genetic disease characterized by increased risk for bone marrow failure and cancer. FA is caused by mutation in one of 23 genes. The FA proteins function together in the FA pathway to repair damaged DNA and to maintain genome stability. In recent years, the prevalence of central nervous system (CNS) abnormalities among FA patients has been increasing. These CNS defects include cerebellar hypoplasia, hydrocephalus, cerebellar lesions with inflammation, developmental delay, and early onset cognitive decline. This constellation of neurological symptoms is referred to as Fanconi Anemia Neurological Syndrome (FANS). The molecular origins of FANS are unknown.

Recent omics data from our laboratory suggests that the FANCD2 protein plays an important role in nervous system development and maintenance. ChIP-seq analysis has revealed that the FANCD2 protein binds to several transcriptionally active large neuronal genes under conditions of replication stress, including genes that function in neuronal differentiation, migration, and cell-cell adhesion. Many of these genes are linked to neuropsychiatric and neurodevelopmental disorders including schizophrenia, autism, and intellectual disability.

To study the role of the FA pathway in nervous system development and maintenance, we have turned to the model

nematode *Caenorhabditis elegans*; *C. elegans* has an exceptionally well-characterized nervous system with exactly 302 neurons and nervous system connectivity has been anatomically mapped at high resolution. Quantitative behavioral analysis of deletion strains of the *C. elegans FANCD2* ortholog, *fcd-2*, has uncovered significant changes in speed, bending angle, and moving wavelength compared to wild-type animals, suggesting a defect in motor neuron circuitry. Using the aldicarb assay to analyze the function of cholinergic (ACh) neurons, we have determined that *fcd-2* mutant animals paralyze at slower rates than wild-type animals under conditions of replication stress, suggesting a defect in the ACh motor neuron circuitry. We have also generated *fcd-2* mutant animals expressing GFP-labeled ACh and dopaminergic (DA) neurons and have uncovered significant differences in numbers of both ACh and DA neurons in *fcd-2* animals compared to wild-type animals. Taken together, our results strongly support an important role for the FCD-2 protein and the FA pathway in nervous system development and function, possibly providing key insight into the molecular origins of FANS.

14 **Mechanisms of photoreceptor stabilization upon chronic vitamin A-deprivation** Deepshe Dewett[1], Khanh Lam-Kamath[2], Joe Bunker[3], Fareeha Syeda[3], Mukesh Kumar[4], Andrej Shevchenko[5], Maryam Labaf[3], Kourosh Zarringhalam[3], Jens Rister[3] [1]Biology, UMASS Boston, [2]Umass Boston, [3]UMass Boston, [4]CST, [5]Max Plank Institute

Vitamin A (vitA) is crucial for the synthesis of the light-sensing pigments and normal photoreceptor morphology. Chronic vitA deficiency results in photoreceptor death and blindness. In *Drosophila melanogaster*, chronic vitA deprivation impairs visual pigment synthesis and damages photoreceptor morphology without inducing photoreceptor death. We hypothesized that an unknown mechanism stabilizes these vitA-deficient photoreceptors. To elucidate this stabilizing mechanism and identify novel factors responding to vitA deficiency, we employed transcriptomics, GeLC-MS/MS mass spectrometry (global proteomics), and MS-Western (targeted absolute protein quantification) in vitA-deficient and vitA-sufficient retinas.

Notably, we identified the transmembrane protein Mps (‘Major photoreceptor stabilizer’) as the most upregulated protein (~140-fold) and the second-most upregulated transcript (~25-fold) in response to chronic vitA deficiency. Immunohistochemistry revealed the localization of Mps in the damaged light-sensing compartments of the photoreceptors (rhabdomeres). Mps stabilized the damaged membranes, since vitA-deficient *mps* null mutants displayed completely unraveled rhabdomere membranes with abnormal invaginations.

To elucidate the molecular mechanism by which Mps stabilizes damaged photoreceptors, we performed a yeast-two-hybrid screen and identified the major photoreceptor scaffolding protein InaD (Inactivation no afterpotential D) as an interaction partner of Mps. In addition, we found that Mps interacts with InaD in a vitA-independent manner during pupal development of the rhabdomeres.

In summary, we identified the transmembrane protein Mps as a novel protein that responds to chronic vitA deficiency and stabilizes the light-sensing compartments by interacting with the major scaffolding protein InaD. These findings offer mechanistic insights into the ocular response to environmental stresses and may inspire novel therapeutic approaches for treating eye diseases.

15 **Transcriptomic landscape of *DEAF1* mutations in zebrafish models of Autism Spectrum Disorder** Mary E.S. Capps[1], Anna J Moyer[1], Claire Conklin[2], Verdion Martina[2], Emma Torija[3], Summer B Thyme[1] [1]UMass Chan Medical School, [2]University of Alabama at Birmingham, [3]University of Alabama

Hundreds of human mutations have been linked to Autism Spectrum Disorder (ASD), but the impacts of these mutations on vertebrate neural development are unclear. The Thyme lab has generated more than 20 zebrafish lines with mutations in orthologs of human genes that increase risk for ASD. In humans with ASD, *DEAF1* is more likely to contain missense than protein-truncating variation compared to other ASD-associated genes. Thus, we generated a loss-of-function mutation and two patient-specific point mutations in zebrafish *deaf1*. We examined brain activity and morphology with phospho-Erk mapping methods and conducted a multi-day larval behavioral study examining sleep and responses to sensory stimulation. All three mutants have a reduced volume in their midbrain, with the loss-of-function displaying a more severe reduction than the two point mutations. Only the line with the C207Y mutation had altered behavior, with movement at night and response to dark flashes being affected. To define how these mutations affect the earliest stages of neurodevelopment, we conducted bulk RNA-seq on 2 days post-fertilization (dpf) larval zebrafish heads. To identify direct targets of deaf1, we focused on a subset of DEGs with DEAF1 binding motifs or ChIP-Seq peaks in their promoters. Notably, several genes, including *khk*, *cdkn1bb*, *creb1b*, *nfyal*, and *atxn7l1*, showed dysregulation across mutants and have DEAF1 ChIP-Seq peaks in human K562 cells. An association of DEAF1 with NF-Y, a trimeric transcription factor, was also observed, with NF-Y binding motifs enriched in the promoters of *deaf1*-regulated genes. Our analysis revealed that the point and loss-of-function mutations in *deaf1* resulted in both shared and distinct transcriptomic changes. In particular, the C207Y mutants exhibited DEGs related to eye development, which could play a role in their altered visual behaviors. Functional analysis of the transcriptome in 6 dpf loss-of-function mutants revealed

downregulation of genes expressed in specific brain regions, including the thalamus, hypothalamus, and telencephalon. These transcriptomic changes could reflect altered development of neurons in these areas, corroborating our microscopy-based brain structure analysis. This study advances our understanding of how mutations in *deaf1* impact early neurodevelopment and sets the stage for future therapeutic development by defining druggable downstream target proteins or pathways in *DEAF1*-related syndromes.

16 **MARRVEL and ModelMatcher: publicly available web services that facilitate collaborative research on rare diseases** Shinya Yamamoto[1,2], Seon-Young Kim[2,3], Michael F Wangler[2,3], Hugo J Bellen[2,3] [1]Department of Molecular and Human Genetics, Baylor College of Medicine, [2]Texas Children's Hospital, [3]Baylor College of Medicine

Diagnosis and therapeutic research of rare diseases require integration of diverse data sets as well as close collaborations between clinicians and scientists. To identify disease-causing genes and variants, clinicians must collect information from various databases such as previously identified disease-gene relationships, population genomic datasets and multiple variant pathogenicity prediction algorithms in addition to the patients' phenotype and genotype information. In addition, if an individual is suspected to have a novel genetic disorder, collecting information associated with the candidate human gene and its orthologs in model organisms, such as mouse, fruit fly, and yeast, becomes essential. For therapeutic research, it is critical to effectively identify collaborative scientists who have expertise in specific genes, biological pathways, or experimental paradigms. However, this information is scattered throughout the internet and is difficult to access comprehensively. Moreover, some valuable information, such as unpublished scientific data regarding poorly characterized genes, is not accessible. To overcome these barriers, we have been developing a suite of bioinformatic tools that are publicly accessible. **MARRVEL** (Model organism Aggregated Resources for Rare Variant ExpLoration, https://marrvel.org/), was designed to integrate many human genomic and genetic databases with various model organism databases to facilitate rare disease diagnosis. **ModelMatcher** (https://www.modelmatcher.net/), is a matchmaking service that was designed to facilitate collaborations between basic scientists with expertise and/or interest in specific genes with clinicians, patients, caregivers, and other stakeholders.

17 **Genome-wide association studies of nephrolithiasis and water transport of renal tubule in *Drosophila melanogaster*** Keqin Li, Shireen Davies, Julian Dow School of Molecular Biosciences, University of Glasgow

Nephrolithiasis, or kidney stones, affects around 10% of adults worldwide. Both genetics and unique environmental factors contribute to the risk of kidney stones. Despite considerable research effort, the lack of understanding about the mechanisms by which stones are formed and of experimentally tractable models hinders the prevention and treatment of nephrolithiasis. The *Drosophila* renal tubule is genetically and functionally comparable to human kidney, making it a suitable translational model of human nephrolithiasis. To identify novel genes involved in the formation of kidney stones, the amount of stone concretion was measured in the renal tubules of ~190 *Drosophila melanogaster* Genetic Reference Panel lines fed on a lithogenic diet. In addition, the capacity of the dissected tubules to secrete fluid was quantified, as insufficient urine volume is thought to be a risk factor for kidney stones. Significant differences were observed both in the percentage of lumens with stone inclusions, which varied between 0 to 21%, and in fluid secretion rates, showing a up to 6.2-fold difference between lines. Using about 2 million common single nucleotide polymorphisms, genome-wide association analyses for the percentage of stone area and fluid secretion were performed. These analyses revealed considerable genetic variation, although no correlation was found between these quantitative traits. A membrane transporter, *CG3380*, with a high expression level in renal tubules was identified by preliminary association analysis of nephrolithiasis, and its knockdown reduced the formation of kidney stones induced by sodium oxalate. Gene Ontology analysis suggested that genes involved in microvilli are important for fluid transport. *Fasciclin3* (*Fas3*), a cell adhesion molecule identified by association analysis, is required for the integrity of renal tubules. The *Drosophila* renal tubules are comprised mainly of principal cells and secondary intercalated stellate cells. In principal cells, loss of *Fas3* resulted in impaired cellular junctions and inflated abdomens, a marker of renal insufficiency. This dysfunction of tubule cellular junctions resulted in increased kidney stone formation and reduced lifespan. In addition, *Fas3* inhibition in stellate cells affected their number and morphology with reduced fluid secretion of tubules. Together, our study provides the first genome-wide association of renal function in animal models and identifies candidate loci as therapeutic targets.

18 **Piwi regulates the usage of alternative transcription start sites in the *Drosophila* ovary via interaction with the FACT complex component Ssrp** Jiaying Chen[1], Na Liu[2], Haifan Lin[2] [1]Genetics, Yale University, [2]Cell Biology, Yale University

Alternative transcription initiation, which refers to the transcription of a gene from different transcription start sites (TSSs), is prevalent in mammalian systems and has important biological functions such as regulating the structure of resulting mRNA and proteins. Although transcriptional regulation has been extensively studied, the mechanism that selects one TSS over others in a gene is still poorly understood. Using the cap-analysis gene expression sequencing (CAGE-seq) method, we identified 87 genes

with altered TSS usage (ATU genes) in Piwi-deficient *Drosophila* ovaries. Piwi differentially regulated TSS usage of ATU genes in germ cells and somatic cells in ovaries as well as in cultured ovarian somatic cells (OSCs). ChIP-seq and CUT&RUN experiments revealed that Pol II initiation and elongation at TSSs of ATU genes were affected in germline-*piwi*-knockdown ovaries and *piwi*-knockdown OSCs. Furthermore, we identified several novel Piwi interactors by Piwi-co-immunoprecipitation of the nuclear fraction of fly ovaries followed by mass spectrometry. Among them, Ssrp is a core component of the FACT complex that acts to reorganize nucleosomes. Temporally controlled knockdown of *Ssrp* affected TSS usage of ATU genes whereas overexpression of *Ssrp* partially rescued the dysregulated TSS usage of ATU genes in *piwi* mutant ovaries. Based on these findings, we propose that Piwi interacts with Ssrp to regulate TSS usage in *Drosophila* ovaries by affecting Pol II initiation and elongation.

19 **Transcriptional adaptation and genetic compensation, from *C. elegans* to mammals** Didier Stainier, Christopher M Dooley, Lara Falcucci, Kuan-Lun Hsu, Gabrielius Jakutis, Brian Juvik, Vahan Serobyan, Jordan M Welker, Lihan Xie, Jie Liang, Cansu Cirzi, Nana Fukuda, Y. Charlie Song, Hamzeh Haj-Hammadeh, Mikhail Sharkov, Greta Ebnicher, Pankaj Kumar Department of Developmental Genetics, Max Planck Institute for Heart and Lung Research

Each human genome has been reported to contain approximately 100 loss-of-function variants, with roughly 20 genes completely inactivated. Some of these completely inactivated genes are essential genes, and yet they are present in a homozygous state in apparently healthy individuals. This totally unexpected lack of phenotype has also been observed in commonly studied model organisms from yeast to mammals. Various hypotheses have been proposed to explain these findings including Genetic Compensation (GC). GC manifests itself as altered gene/protein expression, or function, which leads to a wild-type-like phenotype in homozygous mutant or heterozygous individuals who would be predicted to exhibit clear defects. Traditionally, GC has been thought to involve protein feedback loops such that if one component of a regulatory pathway is deficient, a compensatory rewiring within a network or the activation of a functionally redundant gene occurs. However, not every major regulatory network has evolved to incorporate such complex features. Another mechanism of GC is the newly identified process of Transcriptional Adaptation (TA): some deleterious mutations, but not all, trigger the transcriptional modulation of so-called adapting genes. Depending on the nature of these adapting genes, GC can occur. Notably, unlike other mechanisms underlying genetic robustness, TA is not triggered by the loss of protein function.

We discovered TA while trying to understand the phenotypic differences between knockout (mutant) and knockdown (morphant) zebrafish embryos. Further studies identified additional examples of TA in zebrafish as well as examples in *C. elegans* and in mammalian cell lines. By generating and analyzing several mutant alleles for these genes, including non-transcribing alleles, we found that the mutant mRNA is required to trigger TA. Based on these and other data, we hypothesize that all mutations that cause mutant mRNA degradation can trigger TA. The current model is that mutant mRNA degradation fragments translocate back to the nucleus where they modulate gene expression. Key questions about TA include the identity of the adapting genes and the mechanisms underlying their transcriptional modulation. This presentation will go over our published and unpublished data on TA in several model systems including zebrafish, *C. elegans*, *Neurospora*, and mammalian cells in culture.

20 **Relative enhancer-promoter positioning tunes the kinetics of enhancer-mediated transcriptional activity** Emilia A Leyes Porello, Bomyi Lim, Robert T Trudeau, Emma Dreispiel Juan Chemical and Biomolecular Engineering, University of Pennsylvania

In multicellular eukaryotes, the linear distance between enhancers and promoters ranges from tens to hundreds of kilobases. While the long-range interactions have been studied with bulk genomics assays and some fixed and live imaging approaches, short and intermediate range interactions – particularly the dynamic nature of their contact and resulting transcriptional behaviors – have not been thoroughly characterized . In this study, we probe the regulatory mechanisms underlying enhancer-promoter (E-P) interactions and resulting transcriptional activity at intermediate length scales using early Drosophila embryos. We implemented reporter constructs which systematically modulate various parameters of relative E-P positioning and enhancer strength at distances ranging from 0 kb to 12.6 kb. We also employed the MS2/MCP live-imaging system which enables single cell, real-time quantification of transcriptional activity at the target locus. We find that increasing E-P distances delays the activation of post-mitotic transcription and results in decreased mRNA production per nucleus as distance increases. Strikingly – and in stark contrast to canonical understanding – we also find that placing the enhancer downstream of the promoter and reporter elements drives significantly less transcription with mRNA production falling below the trend set by increasing E-P distance . The downstream positioning of the enhancer does not differentially affect transcriptional activation kinetics with these constructs still fitting the trend of delayed activation with increasing E-P distance. Additionally, quantifying the time spent in the active state (per nucleus) after initial activation, we find that the enhancer downstream subset yielded distinctly diminished activity levels compared to the enhancer upstream subset. These results suggest that increasing E-P distance increases the search space for the elements to find each other thus delaying initiation of transcription; however, once transcription is active and the E-P interaction is established, the linear distance does not seem to affect stability of the active

transcriptional state. Conversely, enhancer positioning relative to the promoter does not influence initiation of transcriptional activity but significantly affects the stability of the active state. These findings suggest there exist additional layers to enhancer-mediated transcriptional regulation that depend on the genomic context within which these elements operate.

21 **Non-additive genetic components contribute significantly to population-wide gene expression variation** Andreas Tsouris[1], Gauthier Brach[1], Joseph Schacherer[1], Jing Hou[2] [1]University of Strasbourg/CNRS, [2]Université de Strasbourg/CNRS

Gene expression variation, an essential step between genomic variation and phenotypic landscape, is collectively controlled by local (*cis*) and distant (*trans*) regulatory changes. Nevertheless, how these regulatory elements differentially influence the heritability of expression traits remains unclear. Here, we bridge this gap by analyzing the transcriptomes of a large diallel panel consisting of 323 unique hybrids originated from genetically divergent yeast isolates. We estimated the broad- and narrow-sense heritability across 5,087 transcript abundance traits and showed that non-additive components account for 36% of the phenotypic variance on average. By comparing allelic expression ratios in the hybrid and the corresponding parental pair, we identified regulatory changes in 25% of all cases, with a majority acting in *trans*. We further showed that *trans*-regulation could underlie coordinated expression variation across highly connected genes, resulting in significantly higher non-additive variance and most likely in some of the missing heritability of gene expression traits.

22 **Chromatin regulatory networks underlying coordinated synaptic gene expression** James Kentro[1], Gunjan Singh[1], Tuan Pham[2], Justin Currie[3], Erica Larschan[1], Kate O'Connor-Giles[4] [1]Molecular Biology, Cell Biology, and Biochemistry, Brown University, [2]Center for Computational Molecular Biology, Brown University, [3]Brown University, [4]Neuroscience, Brown University

During nervous system development, neurons construct intricate circuits that transmit information by passing signals through synaptic connections. These synaptic connections are formed by hundreds of genes encoding proteins for scaffolding, cell adhesion, ion transport, neurotransmitter synthesis and release, signal reception, and more. We have found that hundreds of *Drosophila* synaptic genes share a developmental temporal pattern of expression, with peaks during periods of peak synaptogenesis, that is consistent with coordinated regulation. However, no mechanism for coordinating their expression is known. Using motif discovery, analysis of chromatin binding data, and regulatory network predictions from time-series single-cell transcriptomic and chromatin accessibility datasets, we have identified a candidate gene regulatory network involving several TFs known to interact with chromatin remodelers that may temporally coordinate expression of synaptic genes through opposing influences of repression and activation. In the nervous system, we observe a correlation between the timing of initial synaptic gene transcription and substantial increases in chromatin accessibility at synaptic gene promoters that supports a mechanism of coordination through control of the chromatin environment. We hypothesize that Deaf1, linked to intellectual disability and seizures in humans, interacts with pioneer factor Clamp to negatively regulate synaptic gene expression during periods of low transcription. Consistently, upon loss of either TF, we find an upregulation of synaptic gene transcription. Strikingly, this corresponds to increased glutamatergic synapse formation at neuromuscular junctions. Together, these data support a repressive role for both TFs at synaptic genes during normal development. We are also performing ATAC-seq in the absence of these factors to determine their impact on chromatin accessibility at synaptic gene promoters and potential enhancer sites. We predict that this gene regulatory network coordinates expression of pan-neuronal and subtype-specific synaptic genes upstream of the terminal selectors that establish neuronal identity by controlling chromatin accessibility to coordinate the development of shared traits with the acquisition of distinct neuronal fates.

23 **Interallelic gene regulation promotes robustness and evolvability** Noa O Borst[1], Timothy Fuqua[2], Fabian Ruperti[1], Justin Crocker[1] [1]EMBL, [2]University of Zurich (UZH)

Enhancers drive complex spatiotemporal patterns of gene expression during development, and their evolution is an important driver of morphological and phenotypic diversification. Recently, we found that random point mutations in the *E3N* enhancer of *shavenbaby* in *D. melanogaster* resulted in changes in the level, timing, or location of its activity, suggesting that the enhancer is densely encoded with regulatory information. To explore how such dense encoding of regulatory information might constrain the evolution of this enhancer, we systematically explored all the possible evolutionary paths between the modern-day *E3N* enhancers of *D. melanogaster* and *simulans*. Consistent with our previous results, we observed extensive higher-order epistatic interactions between genetic variants across these evolutionary trajectories, with many paths reducing transcriptional outputs. Strikingly, interchromosomal interactions between heterozygous enhancer alleles resulted in synergistic or antagonistic effects on gene expression, a phenomenon commonly recognized as dominance. Furthermore, we found evidence that these transvection-like interactions are reinforced through transcriptional hubs. Beyond the previously established role of transcriptional hubs in developmental robustness, we propose that these regulatory interactions concurrently promote evolutionary robustness and enable the exploration of novel phenotypes. Consequently, interallelic interactions may provide an additional layer for natural selection to act on in regulatory evolution.

24 **The role of post-transcriptional gene regulation in adaptation to stress** Pieter Spealman[1], Carolina de Santana[2], Titir

De[1], David Gresham[1] [1]New York University, [2]Laboratório de Microbiologia Ambiental e Saúde Pública, Universidade Estadual de Feira de Santana

The importance of divergence in gene expression has been a central concern of evolutionary biology for nearly 50 years. While the majority of research has focused on changes in transcription, recent research suggests that post-transcriptional regulation may play a vital role in adaptive gene expression.

Using a combination of RNA-seq, ribosome profiling, and mass-spectrometry we identify divergence in abundance across three levels of gene expression between 4 strains of experimentally evolved yeast (Saccharomyces cerevisiae) and their ancestor (derived from FY4). Strains were evolved using long-term evolution experiments for 150 and 250 generations in glutamine-limited media conditions; all evolved strains have increased fitness and genomic amplifications that include the GAP1 locus, a general amino-acid permease.

By normalizing each level of expression by the abundances observed at the previous level (ie. RNA/DNA, RPF/RNA, MS/RPF) we identify specific levels of regulation. We find a mean of 1116 genes per strain have significantly different RNA/DNA ratios, a mean of 389 RPF/RNA ratios, and a mean of 153 MS/RPF ratios. We find that a substantial number of genes (mean 25%) with significantly different RNA/DNA ratios are significantly buffered at later levels of expression. We find a mean of 32% of genes are significantly different only at the post-transcriptional level.

We find that translationally regulated genes frequently (mean 27%) have upstream open reading frames (uORFs) and that these are also frequently (mean 35%) enriched in SSD1 binding sites. SSD1 is a known stress-responsive RNA binding protein that has been shown to localize to 5' UTRs and alter translation. An analysis of post-translational buffering finds that few buffered genes are part of protein complexes (mean 8.8%), suggesting that post-translational buffering mechanisms may not rely on stoichiometric imbalances.

Taken together, these results point to extensive changes in gene expression that accrue over the course of adaptation to glutamine-limitation and genomic amplification. They highlight the role of post-transcriptional buffering of transcriptional divergence as well as divergence in expression derived from post-transcriptional mechanisms. They also point towards a novel mechanism of uORF mediated translational regulation driven by stress-responsive RNA binding protein, SSD1.

25 **Social regulation of intergenerational signaling via a defined chemosensory pathway** Jadiel Wasson[1], Susan Mango[2] [1]New York University, [2]University of Basel

Classically, inheritance was believed to be restricted to the passage of information from parent to progeny in the form of genetically encoded material. It has become appreciated that other types of information, including that which informs about the environment, can be passed between generations. However, the mechanisms behind how this information can be both passed on and interpreted by the embryo remain unknown. Recently, we have identified a previously unknown pathway for intergenerational communication that links neuronal responses to maternal provisioning in *C. elegans.* Here, a chemosensory signaling pathway responsive to social cues initiated in the mother alters the pool of maternally provided factors that modulates gene silencing in progeny. This intergenerational signal transmission depends on specific chemosensory neurons and neuronal FMRFamide (Phe-Met-Arg-Phe)–like peptides including FLP-21. Parental FLP-21 signaling dampens oxidative stress resistance and promotes the deposition of mRNAs for translational components in progeny, which, in turn, reduces gene silencing. Furthermore, overexpression of FLP-21 has the opposing effect on gene silencing in progeny suggesting that the levels of FLP-21 signaling in mothers *influences* embryonic stress phenotypes. We are currently investigating different aspects of this maternal signaling pathway including the neurocircuitry, key molecular components, and tissue-specific requirements to identify the relay from environmental social cues to modulation of embryonic gene silencing. To this end, we have identified key receptors that are required for this relay from parental environment to progeny response. Taken together, we hypothesize that loss of social cues in the parental environment represents an adverse environment that stimulates stress responses across generations. This work demonstrates how alterations to chemosensory signaling pathways can have long range consequences via changes in not only what mothers provide to their young, but also how resulting progeny modulate their gene expression changes in response to a challenge. Ultimately, this work will lead to a clearer understanding of the mechanisms involved in cross-generational signaling between mother and progeny.

26 **Repeated, rapid origins of incompatibility in the Mimulus guttatus species complex: the role of shared variation in repeated speciation** Jenn Coughlan Ecology & Evolutionary Biology, Yale University

How new species originate and persist in nature is a fundamental question in evolutionary biology. Although genetic incompatibilities underlying hybrid sterility and inviability can play a central role in speciation, much remains unknown about the evolutionary forces ultimately responsible for incompatibility alleles. Much classical theory highlights the role

of intra-genomic conflict in the origin of intrinsic crossing barriers, yet we are only beginning to empirically test these hypotheses. Here, I combine a series of range-wide crossing surveys, quantitative genetics, and population genomics to dissect the repeated origin of hybrid seed inviability in a group of closely related, rapidly speciating wildflowers; the *Mimulus guttatus* species complex. I find multiple, phenotypically cryptic lineages within this group that are strongly reproductively isolated by hybrid seed inviability, suggesting that this barrier has evolved rapidly and repeatedly. By combining hybrid phenotypes with genetic mapping, I test whether intra-genomic conflict between maternal and paternal interests in resource partitioning to offspring can explain patterns of rapid and repeated evolution of hybrid seed inviability. I find that patterns of hybrid seed inviability strongly support a role of parental conflict in driving speciation, and subsequent meta-analyses suggest that parental conflict may play an important role in angiosperm diversity more broadly. Intriguing, while hybrid seed inviability between *M. guttatus* and several cryptic lineages appears to have evolved repeatedly, crosses between lineages with putatively independent origins of this barrier are viable, suggesting that the alleles underlying these two incidences of hybrid seed inviability are in some way complementary. Using a classical complementation test, I then ask whether the alleles causing independent origins of hybrid seed inviability are shared. In total, I find the rapid and repeated evolution of hybrid seed inviability in this group suggests that parental conflict may play an important and generative force in speciation. Moreover, complex patterns of incompatibility point to a complex genetic origin; potentially involving a combination of hybridization, ancestral polymorphisms and/or de novo mutation in the repeated origin of reproductive isolation.

27 **A programmed Mendelian violation maintains heterozygosity in a parthenogenetic ant** Kip D Lacy, Daniel J.C. Kronauer Laboratory of Social Evolution and Behavior, The Rockefeller University

Parthenogenesis arises sporadically across the animal phylogeny, evolving via mutations that perturb sex and meiosis. The underlying mechanisms are rarely understood, but might provide insights into how meiosis works and how asexuality evolves. Here, we investigate parthenogenesis in the clonal raider ant, *Ooceraea biroi*, which produces diploid offspring via the fusion of two haploid products from a single meiosis. This process should cause rapid genotypic degeneration due to loss of heterozygosity, which results if crossover recombination is followed by random (Mendelian) segregation of chromosomes. However, by comparing whole genomes of mothers and daughters, we show that loss of heterozygosity is exceedingly rare, raising the possibility that crossovers are rare or absent from *O. biroi* meiosis. Using a combination of cytology and whole genome sequencing, we show that crossovers occur regularly, but loss of heterozygosity is avoided because crossover products are faithfully co-inherited. This results from a programmed violation of Mendel's law of segregation, such that crossover products co-segregate rather than segregate randomly. This discovery highlights an extreme example of cellular "memory" of crossovers, which could be a common yet cryptic feature of chromosomal segregation.

28 **Adaptive piRNA pathway tuning tames sex- and lineage-specific selfish genes** Peiwei Chen[1], Katherine C Pan[1], Eunice H Park[1], Grace YC Lee[2], Alexei Aravin[1] [1]California Institute of Technology, [2]University of California, Irvine

Selfish genetic elements subvert fair Mendelian inheritance for their own benefit at the expense of the host, causing intragenomic conflicts that must be resolved to protect host reproduction. Although selfish genes differ between sexes and across lineages, how sex- and lineage-specific selfish genes are tamed remains mostly unknown. Here, using the silencing of *Stellate*—a recently evolved, selfish gene family only active in *Drosophila melanogaster* male germline—as a readout, we conducted an *in vivo* RNAi screen and discovered a novel genome defense factor required for *Stellate* silencing that we named Trailblazer. Contrary to all known protein components of the genome-defending piRNA pathway in flies, Trailblazer is essential for male but not female fertility. By enhancing the expression of cytoplasmic PIWI proteins, Aub and AGO3, Trailblazer enables the destruction of *Stellate* transcripts, whose abundance in the male germline exceeds that of all transposons by two orders of magnitude. While *trailblazer* is conserved outside *D. melanogaster*, it is subject to lineage-specific positive selection, and efficient *Stellate* silencing requires Trailblazer's adaptive changes in recent evolution. Hence, sex- and lineage-specific selfish genes have spurred genetic innovations in genome defense, which tunes ancient defense machinery adaptively to nullify contemporary intragenomic threats—a strategy we suspect is recurrently employed for genome defense.

29 **Genetic conflicts shape rapid evolution of young *Drosophila* protamines** Ching-Ho Chang[1], Isabel Mejia Natividad[1], Aditi Kishore[2], Harmit S Malik[1] [1]Fred Hutchinson Cancer Center, [2]University of Washington

Eukaryotes commonly use histones for packaging their genomes, but many animal species utilize positively charged protamines for tighter genome packaging in sperm. Although histones are ancient and highly conserved, protamines exhibit rapid evolution and positive selection across different animals. The prevailing hypothesis attributes this accelerated evolution to sexual selection favoring competitive sperm morphology, evident from densely packed sperm nuclei. Our previous phylogenomic surveys in 78 *Drosophila* species introduce the idea that genetic conflicts between sex chromosomes might also contribute to the rapid evolution of protamines. Here, we focus on *Mst77F*, a young, subgenus-specific protamine gene that is rapidly evolving but essential for male fertility in *D. melanogaster*. Remarkably, *Mst77F* has undergone 18 duplications on the

Y chromosome of *D. melanogaster*. We hypothesize that autosomal, ancestral Mst77F suppresses X-Y genetic conflicts, while its expanded Y-linked counterparts amplify it. To test this, we conducted gene-swap analyses in *D. melanogaster* to dissect the functional divergence of the Mst77F homologs. We showed that replacing Mst77F-mel with an ortholog from a distantly related species, *D. ananassae*, leads to male sterility. Conversely, replacements of orthologs from closely related species like *D. yakuba* resulted in low male fertility, marked by male-biased progeny due to a scarcity of X-bearing sperm. Furthermore, overexpression of Y-linked *Mst77F* copies in *D. melanogaster* produced a similar phenotype of male-biased progeny. Our findings demonstrate that the rapid evolution of *Mst77F* is driven by its role in suppressing or enhancing X-versus-Y sperm killing. We further suggest that, in addition to sperm competition, genetic conflicts may propel the paradoxical but universal rapid evolution of protamine genes.

30 **Telomere protein coevolution preserves chromosome end protection** Sung-Ya Lin[1], Hannah Futeran[1], Mia T. Levine[1,2] [1]Department of Biology, University of Pennsylvania, [2]Epigenetics Institute, University of Pennsylvania

The terminal ends of eukaryotic chromosomes threaten genome integrity. If left unprotected, inappropriate DNA repair fuses chromosomes end-to-end. Specialized telomere capping complexes protect chromosome ends from this catastrophic DNA repair. Chromosome end protection is essential and deeply conserved across eukaryotes, yet multiple telomere capping proteins evolve adaptively. *Drosophila* has two such proteins, HOAP and HipHop. We previously showed that the end protection protein, HOAP, evolved to restrict telomeric retrotransposons, while the evolutionary pressure on HipHop is unknown. HipHop physically interacts with HOAP and recruits it to the telomere, raising the possibility that HipHop coevolves with HOAP to preserve end protection. To test this hypothesis, we replaced the native *D. melanogaster hiphop* with a highly diverged version from its close relative, *D. yakuba* (*hiphop[yak]*). We discovered that *D. melanogaster* flies encoding only HipHop[yak] were homozygous lethal due to rampant telomere fusions. We also found that HipHop[yak] localized robustly to these telomeres but the *D. melanogaster* HOAP (HOAP[mel]) failed to localize, suggesting that HOAP requires its conspecific HipHop to localize and preserve end protection. To test this prediction, we swapped *HOAP[yak]* into *hiphop[yak]* flies. Excitingly, HOAP[yak] localizes to the *hiphop[yak]* telomeres and rescues both end protection and viability, consistent with HipHop-HOAP coevolution. Furthermore, the viability of *hiphop[yak]/+* heterozygous flies but lethality of *hiphop[yak]* homozygous flies suggests that one copy of *hiphop[mel]* is sufficient to recruit HOAP[mel] to the telomeres and preserve end protection. This inference, however, fails to account for a second, puzzling observation: *hiphop[yak]/+* heterozygous females, which encode one copy of the native *D. melanogaster* HipHop, are severely subfertile. To probe this, we studied the subfertile *hiphop[yak]/+* females. Their embryos die at the first embryonic mitosis, a phenotype caused by paternal chromosome-specific telomere dysfunction. Wildtype, sperm-deposited paternal telomeres lack HipHop and require *de novo* loading of maternally provisioned HipHop, suggesting that maternal HipHop[yak] may interfere with the loading of maternal HipHop[mel] and ultimately impede end protection. Importantly, embryonic lethality is rescued by swapping in *HOAP[yak]*. These results are consistent with HipHop-HOAP coevolution preserving distinct telomere packaging events during development. We propose that HOAP evolution to restrict retrotransposons triggers HipHop evolution to maintain their protein-protein interaction. These data implicate a selfish element-initiated “evolutionary cascade” that sculpts fundamental cellular processes vital for viability and fertility.

31 **Post-insemination sexual selection in males indirectly masculinizes the female transcriptome** Katja Kasimatis[1], John Willis[2], Patrick Phillips[2] [1]University of Virginia, [2]University of Oregon

Sex-specific regulation of gene expression is the most plausible way for generating sexually differentiated phenotypes from an essentially shared genome. However, since genetic material is shared, sex-specific selection in one sex can have an indirect response in the other sex. From a gene expression perspective, this tethered response can move one sex away from their wildtype expression state and impact potentially many gene regulatory networks. Here, using experimental evolution in the model nematode *Caenorhabditis elegans,* we explore the coupling of direct sexual selection on males with the transcriptomic response in females over microevolutionary timescales to uncover the extent to which post-insemination reproductive traits share a genetic basis between the sexes. We find that differential gene expression is driven by female ancestral or evolved generation alone and that male generation has no impact on changes in gene expression. Almost all differentially expressed genes were downregulated in evolved females. Moreover, 80% of these gene were located on the X chromosome and have wildtype female-biased expression profiles. Changes in gene expression profiles were likely driven through *trans*-acting pathways that are shared between the sexes. We found no evidence that the core dosage compensation machinery was impacted by experimental evolution. Together these data suggest masculinization of the female transcriptome driven by direct selection on male sperm competitive ability. Our results indicate that on short evolutionary timescales sexual selection can generate sexual conflict in expression space.

32 **The impact of genomic autoimmunity across the Drosophila genus** Justin Blumenstiel[1], Martina Dalikova[1], Jeffrey Vedanayagam[2] [1]University of Kansas, [2]University Texas San Antonio

Transposable elements threaten genome integrity and small RNA based systems of genomic immunity have evolved to ward against this damage. However, as observed in other immune systems, there is a cost of genome defense when the distinction between self and non-self is blurred. This results in off-target gene silencing, which can be considered a form of genomic autoimmunity. Here we describe several systems of genomic autoimmunity that differ in how off-target gene silencing by piRNA is triggered and transmitted across generations. In one case, genic silencing can be maintained in a transgenerational manner. However, this isn't the case in the second example. The mechanisms underlying these differences are poorly understood. Across the genus Drosophila there is significant variation in transposable element abundance, and we hypothesized that this would lead to significant variation in the degree of genomic autoimmunity. Surprisingly, the impact of genomic autoimmunity is remarkably uniform. To test the hypothesis that the primary driver of rapid evolution of the piRNA machinery is driven by an evolutionary arms race, we developed a new maximum likelihood framework for the McDonald-Kreitman test to compare adaptive evolution in the piRNA machinery across species with low and high TE content. Using this approach, we were able to reject the hypothesis that greater genomic TE content leads to increased adaptation in the piRNA machinery. Together, these results question the hypothesis that rapid evolution of the piRNA machinery is driven by an evolutionary arms race but highlight a complex role of genomic autoimmunity in the evolution of the genome defense based on small RNAs.

33 **Cellular and molecular organization of the Drosophila foregut** Haolong Zhu[1,2], William B Ludington[1,2], Allan C Spradling[1,2,3] [1]Embryology, Carnegie Institution for Science, [2]Biology, Johns Hopkins University, [3]Howard Hughes Medical Institute

The animal foregut is the frontline tissue encountered by ingested food, bacteria, and viruses. Efficiently digesting and absorbing nutrients from this intake, while retaining beneficial microorganisms and rejecting pathogenic ones represents a critical task for this gatekeeper tissue and requires coordinative responses of multiple tissues. To delineate these intricate physiological processes, we characterized the adult Drosophila foregut as a model. Major foregut cell types were identified, validated, and examined using scRNAseq and >150 *in vivo* gene reporter fly lines. Transcriptome analysis revealed potential mechanisms that the foregut-associated neuroendocrine cells including the *corpus cardiacum* and *corpora allata* use to coordinate gut activity with nutrition, the microbiome, and circadian cycles. Multiple intestinal cell types differentially express juvenile hormone binding proteins that likely allow them to respond to changes in juvenile hormone produced by the *corpora allata*. In addition to juvenile hormone biosynthetic enzymes, the *corpora allata* expresses circadian clock genes. Studying all the cell types of the proventriculus, the central foregut organ that secretes the peritrophic matrix lining the intestinal tract, provided a detailed view of the layered structure and dynamic character of the peritrophic matrix. Analyzing cell types synthesizing individual peritrophic matrix layers revealed abundant mucin production close to enterocytes, similar to the mammalian intestinal mucosa. Two previously unrecognized cell groups in the posterior proventriculus produce abundant digestive enzymes likely moving posteriorly together with the peritrophic matrix to join ingested food. The esophagus and salivary gland express secreted proteins some of which may line the esophageal surface and generate a foregut commensal niche for specific gut microbiome species including Lactiplantibacillus and Acetobacter strains (Dodge *et al*. 2023). Overall, our study strongly supports the foregut's role as a central coordinator of intestinal activities, immune defense, and microbiome interactions by virtue of its location near the brain, sensory organs, and esophagus, the most frequent site of pathogen invasion. Our results also suggest that the cells, genes, and functions of the foregut as an interacting group of tissues, have been significantly conserved throughout animal evolution.

Dodge *et al*. (2023). Nat. Comm.14:1557.

34 **Spatial patterning regulates neuron numbers in the *Drosophila* visual system** Jennifer Malin, Yen-Chung Chen, Felix Simon, Evelyn Keefer, Claude Desplan Department of Biology, New York University

Neurons must be made in the correct proportions to carry out their functions. In the *Drosophila* visual system, 20 classes of distal medulla (Dm) neurons are specified with characteristic numbers—from 5 to 800 per optic lobe—to regulate the flow of visual information from photoreceptors. Dm neurons are born from a crescent-shaped neuroepithelium called the Outer Proliferation Center, or OPC. During the third larval instar, neuroepithelial cells are converted into neural stem cells called neuroblasts, which express a series of temporally regulated transcription factors that specify distinct neural classes. Additional diversity is produced via the asymmetric division of transit-amplifying Ganglion Mother Cells, which generate one Notch-on neuron and one that is Notch-off. In addition to temporal patterning, the OPC is partitioned based on the spatially-restricted expression of transcription factors and growth factors: Vsx is expressed at the center of the OPC, Optix is expressed in the middle, and Rx is expressed at the tips. Rx can be further divided into two subdomains based on differences in Dpp/Wg expression.

Using a series of genetic fate mapping tools, including single-cell RNA sequencing based techniques, we characterized the

role of spatial patterning in the regulation of neuronal stoichiometry. We found that the relative abundance of a specific Dm subclass is directly proportional to the size of the neuroepithelial domain from which it is born. Although spatial patterning can explain most Dm cell number variation, it is not sufficient to explain differences in less abundant cell types. Mutations in apoptosis genes caused a small increase in cell number, but were not sufficient to account for gross differences in cell type proportions. In contrast, we found that additional spatial patterning subdivides the OPC to generate additional diversity. We found that the Dm neuron Dm8 is born from the anterior ventral 2/3 of the Optix domain, while Dms 1, 4 and 12 are born from the posterior ventral 1/3 of the Optix domain. To generate this subdivision, we found that the morphogen Dpp (BMP) prevents Brinker (Brk) expression to delimit it to a domain that covers Vsx and the posterior 2/3 of the ventral Optix domain, thereby allowing for differential specification of Dm1/4/12 vs. Dm8 in the Optix domain. Inhibition or overexpression of Dpp and/or Brk changed the relative ratios of Dm1/4/12 vs Dm8 cells, suggesting that Dpp can regulate cell number as well as cell fate. Dpp expression also overlaps with the spatial factor Optix, which forms an additional spatial subdomain within the neuroepithelium. Our scRNAseq lineage tracing data shows that Dm12 is born from the area with highest Dpp expression, while Dm1 and Dm4 are born from areas of lower Dpp expression. Altogether, we show that spatial patterning generates differently sized pools of stem cells to generate neurons in distinct proportions.

35 **Protein-intrinsic and extrinsic features regulating pioneer factor-mediated reprogramming** Eliana F Torres Zelada[1], Elizabeth D Larson[1], Hideyuki Komori[2], Meghan M Freund[1], Abby Ruffridge[1], Christine Rushlow[3], Cheng-Yu Lee[2], Melissa Harrison[1] [1]Department of Biomolecular Chemistry, University of Wisconsin-Madison, [2]Department of Cell and Developmental Biology and Life Sciences Institute, University of Michigan, [3]Department of Biology, New York University

Transcription factors coordinate changes in cellular identity by binding DNA to drive gene expression. However, chromatin is a barrier to transcription-factor binding. Pioneer factors are a unique class of transcription factors that bind DNA in the context of nucleosomes, establish regions of accessible chromatin, and define *cis*-regulatory regions to control gene expression. These properties make them instrumental for driving key developmental transitions. Nonetheless, developmental context regulates pioneer-factor function. To investigate the reciprocal relationship between development and pioneer activity, we study the pioneer factor Zelda (Zld). Following fertilization, Zld is required for reprogramming the specialized germ cells to the totipotent cells of the early embryo. Zld is similarly required to promote self-renewal in neural stem cells, neuroblasts (NB), in the larval brain. We showed that Zld occupies unique regions of the genome in NBs and embryos. Indeed, other pioneer factors show cell-type-specific DNA binding. Thus, while pioneer factors bind to nucleosomes, there are features that shape this binding. Zld a 1596 aa protein that includes six zinc fingers (ZnF). A cluster of four zinc fingers (ZnF3-6) are required for binding the canonical DNA motif, CAGGTA. ZnF2 functions as an auto-inhibitory domain in embryos and cell culture, such that mutations in ZnF2 result in hyperactivation of Zld-target genes. By contrast, ZnF2 is necessary to promote the stem-cell fate in the NB lineage. In NBs and cell culture, ZnF2 is required for binding to chromatin regions lacking the CAGGTA motif, but is dispensable for binding chromatin in the early embryo. Using gel shift assays, we showed that ZnF2 mediates binding to DNA containing a G-rich motif, suggesting that ZnF2 may promote DNA binding. Together our data support the model that in the embryo the DNA-binding activity of ZnF2 may inhibit Zld-mediated activation of target genes, which nearly all contain the CAGGTA motif. By contrast, in NBs ZnF2 binding to G-rich motifs is likely essential for function as many NB enhancers lack the canonical motif. Ongoing studies focus on the role of these two DNA-binding domains in regulating the ability of Zld to promote chromatin accessibility and the tissue-specific cofactors that may mediate the differential dependence on these two domains. Our studies will uncover tissue-specific regulation of a paradigmatic pioneer factors and provide insights into how pioneer-factor activity can be regulated to enable rapid transitions in cell fate.

36 **Secretion and Transportation of the Hedgehog Morphogen in *Drosophila*** Pascal Therond[1], Tamas Matusek[1], Sandrine Pizette[1], Catherine Rabouille[2], Laurent Ruel[1], Laurence Lavenant[1] [1]CNRS, [2]Hubrecht Institute/KNAW &UMC

We focus our studies on the conserved family of Hedgehog (Hh) proteins, which carry hydrophobic lipid modifications essential for their correct spreading in the extracellular environment. Various potential extra-cellular carriers for Hh have been suggested, including soluble multimers, long filopodia, extracellular vesicles (EVs), or lipoprotein particles. The individual contribution of these carriers to the morphogen activity of Hh is still unresolved. We recently provided evidence that Hh activity is divided between two functionally distinct Hh extracellular pools, one basal and one apical, which collectively comprise the morphogen gradient (Gore et al., Development 2021). As the regulation of Hh trafficking is likely critical for Hh loading on one of its carriers for extracellular transportation, we choose to study the different intracellular routes followed by Hh in producing epithelial cells. We demonstrated that Hh followed a primary secretion to the apical plasma membrane before its endocytosis for a secondary secretion (D'Angelo et al., Dev. Cell 2015). We further provided evidence that apical microvilli act as a source for an active pool of Hh secreted on EVs to exert long-range signaling function (Matusek et al., Nature 2014; Hurbain et al., Current Biol. 2022). We recently showed that the major pool of intracellular Hh in producing cells is present in a novel compartment (identified by electronic microscopy) which correspond to recycling tubules emanating from endosomes (Pizette et al., J. Cell Sci. 2021). We proposed a model whereby upon primary secretion by the producing cells, a pool of Hh is

re-endocytosed to Rab11-dependent endosomes and recycling tubules to stimulate high-level signaling and disc pouch growth (Pizette et al., 2021). Using the new RUSH method we recently documented the role of two new players, TMED10 and Rab18, which participate in Hh exit from the endoplasmic reticulum (ER; Bar et al., Cell. Mol. Life Sci. 2023). However, our preliminary data suggests that TMED10 and Rab18 participate also in the loading of Hh onto intraluminal vesicles of MVBs, an unforeseen result that drove us to decipher their role in the incorporation of Hh into EVs and subsequent signalling functions. I will present our last data on the biogenesis of Hh-EVs and the role of TMED10 and Rab18 in this process.

37 **Decoding Butterfly Wing Patterning: Single-Cell Multi-Omics Analysis of the Uncharted *WntA/fz2* Signaling Pathway** Anyi Mazo-Vargas Biology Department, Duke University

The *WntA* ligand emerges as a central orchestrator in instructing butterfly wing color patterning, providing a new opportunity to investigate pattern formation in a simple tissue. Previous studies employing genetic mapping in natural populations, in-situ hybridizations, and CRISPR knockouts have unveiled the profound influence of the *WntA* in delineating distinct boundaries within the developing wings of closely and distantly related butterfly species, resulting in striking differences in patterns. Initial findings suggest that the *WntA/fz2* interaction follows a non-canonical signaling pathway. Yet, the cascade of events and the targets within this transduction pathway that underlie spatial differentiation and its connection to scale determination remain poorly understood. In this study, I employed CRISPR/*Cas9* technology to induce gene and regulatory perturbations in the Gulf fritillary butterfly *Agraulis incarnata*. This approach allowed the modulation of the *WntA* pathway in both directions—utilizing null *WntA* alleles (loss-of-function) and a cis-regulatory deletion resulting in *WntA* overexpression (gain-of-function). Through the analysis of single-nuclei RNA and ATAC-seq profiles during the early pupal development stage, I investigate the impact of these signaling shifts on cell-type specific expression and chromatin-accessibility profiles. The findings identified potential candidate genes associated with alterations in the *WntA/fz2* signaling, contributing to the establishment of diverse wing elements. This research illuminates the intricate molecular processes underpinning butterfly wing pattern development and provides insights into the critical genes and regulatory elements shaping this phenomenon.

38 **Transcriptional control of male-specific tail tip morphogenesis in *C. elegans* by DMD-3** Porfirio Fernandez[1], Karin Kiontke[1], David H. A. Fitch[2] [1]Biology, NYU, [2]NYU

Morphogenesis is characterized by cell shape changes and other cell behaviors carried out by 'effector' proteins that must be regulated in time, space, and sex. However, how transcriptional regulators are connected to effectors is poorly understood. To elucidate this link, we use the C.elegans male tail tip. In both sexes, the larval tail tip is pointed. In males only, its shape changes to round during the L4 stage. This Tail Tip Morphogenesis (TTM) is controlled by a transcription factor, DMD-3, which is required and sufficient for TTM. RNA-seq and differential expression (DE) analysis of tail tips isolated from wild-type (WT) and dmd-3(-) males at the L4 stage identified 1154 genes regulated by DMD-3 directly and indirectly. To find direct targets of DMD-3, ChIP-seq on whole males was done. We found 1755 peaks corresponding to 3636 genes and a DMD-3 binding motif. 147 genes were also DE and represent the genes directly regulated by DMD-3 in the tail tip. To confirm the function of the DMD-3 binding motif, we tiled the motif sequence and injected it as a transgene. Males showed defective TTM, while control animals were WT, indicating that the array could sequester DMD-3 protein. To validate DMD-3 targets, GFP was inserted into endogenous loci and expression dynamics were profiled. We then deleted the candidate peak sequence or the DMD-3 binding motif and screened for altered expression or TTM defects. We found 4 genes in which such deletions had an effect: (1) FOS-1 is expressed in the tail-tip of males and other tissues. Abolishing the DMD-3 binding sites resulted in a specific loss of GFP in tail tips and defective TTM. (2) NMY-2 is expressed in the tail tip during TTM and abolishing the peak reduced tail-tip expression. (3) PAN-1 has 2 peaks; deleting one has no effect, while deleting the other results in 10 % of males with defective TTM. Eliminating both peaks recapitulates the pan-1(RNAi) phenotype in both sexes. (4) HMR-1 localizes to adherens junctions and later forms cytoplasmic puncta. Transcriptional reporter assays showed a male-specific delay in hmr-1 expression when the peak was deleted in the promoter construct. Deleting the peak endogenously has little effect on protein expression; however, adults of both sexes displayed the hmr-1(RNAi) phenotype. Taken together, these data suggest that DMD-3 binds DNA to regulate the expression of sex-shared genes to fine-tune male-specific TTM.

39 **Ancient evolutionary origin of a pluripotency-neural crest gene regulatory network in vertebrates** Joshua York[1], Carole LaBonne[2] [1]Northwestern University, [2]Molecular Biosciences, Northwestern University

Neural crest cells are a vertebrate-specific innovation that helped drive the evolution of early vertebrates. A key feature of this stem cell population is its broad, multi-germ layer developmental potential. Here, we investigate the evolutionary origins of neural crest potential by comparing neural crest and pluripotency gene regulatory networks (GRNs) in representatives of both jawless (lamprey) and jawed (*Xenopus*) vertebrates. Using comparative gene expression analyses and transcriptomics we reveal an ancient evolutionary origin of shared components between neural crest and pluripotency GRNs that dates back to the last common ancestor of extant vertebrates. Focusing on the key pluripotency factor pou5, we show that lamprey genome has

a pou5 ortholog that is expressed in animal pole cells, as in jawed vertebrates, but is absent from the neural crest. However, gain-of-function experiments show that both lamprey and *Xenopus* pou5 promote neural crest formation, suggesting that pou5 was lost from the neural crest of jawless vertebrates. Finally, we show that pou5 is essential for neural crest development in jawed vertebrates and that it acquired neural crest-enhancing activity after evolving from an ancestral pou3-like clade that lacks this functionality. We propose that a pluripotency-neural crestGRN was assembled in stem vertebrates and that the multi-germ layer potential of the neural crest evolved by deploying this regulatory program. Using ATAC and ChIP-seq we show that both stem cell populations pou5 partners with SoxB1 factors to control developmental potential.

40 **Ribosome heterogeneity drives early development in zebrafish** Kamena Kostova Carnegie›s Department of Embryology

In zebrafish (Danio rerio), early embryonic development is characterized by remarkable change in the composition of the ribosome, the molecular machine that translates mRNA to protein. The zebrafish oocyte is loaded with a special type of ribosome, called maternal ribosome, that carries out protein production during the early stages of development. During maternal to zygotic transition the embryonic genome becomes transcriptionally active and the assembly of somatic ribosomes begins. These ribosomes function in all tissues and organs of the adult animal. How the assembly of two structurally different types of ribosomes regulates protein production to drive early development is not known.

Here, we report that maternal and somatic ribosomes differ in their 3D structure, location and identity of post translational modifications, as well as interacting partners. We present in vivo and in vitro approaches to measure how maternal and somatic ribosomes differ in their ability to engage mRNAs and synthesize proteins. We use genetic and biochemical approaches to determine how disruption of the tightly orchestrated transition between maternal and somatic ribosomes interferes with normal development in zebrafish. Our work challenges the traditional mRNA-centric view of early development, uncovering how assembly and deposition of ribosomes with altered structure and mRNA specificity regulates protein production to facilitate embryogenesis.

41 **BindCompare: A Novel Platform to Identify and Analyze Coupled Protein-DNA and Protein-RNA Binding Regions** Pranav Mahableshwarkar, Mukulika Ray, Erica Larschan Brown University

Transcription factors (TFs) and RNA binding proteins (RBPs) work synergistically or antagonistically to mediate gene regulation. However, how TFs and RBPs co-interact with DNA and RNA to drive precise gene expression for cell and sex-specific functions remains unclear. While high throughput techniques like CUT&RUN, ChIP-seq, and iCLIP enable the study of protein-nucleic acid interactions, there is a dearth of computational platforms that integrate multiple protein-nucleic acid datasets to understand how different TFs and RBPs regulate precise function at specific genomic locations. We present BindCompare, a novel platform to identify genomic regions where coordinated gene regulation and RNA processing occur by integrating high throughput protein-nucleic acid binding genomic datasets.

BindCompare uses BED files that list where TFs and RBPs bind to DNA or RNA. Optimized algorithms process the RNA/DNA binding sites in one dataset to find overlapping binding domains in corresponding datasets. The search for overlaps occurs in a user-specified scoped region because co-regulatory binding can occur upstream and downstream of the DNA binding site. The visualized results convey the distribution and frequency of overlaps across the scoped region. These techniques can be split across chromosomes to enable users to see if the co-regulatory activity is concentrated in particular domains. To determine the downstream functionality of co-regulatory regions, the app embeds platforms like GProfiler2 and MEME-Suite, enabling rapid gene ontology and motif analysis.

As a proof of concept, we compared the binding of DNA and RNA binding TF CLAMP, Chromatin-linked adaptor for male-specific lethal proteins, and RNA helicase maleless (MLE). Using BindCompare, we showed the first experimental proof of CLAMP binding to DNA and RNA molecules at proximity, indicative of CLAMP's co-transcriptional activity. It was further shown that this mechanism concentrated on the X chromosome. BindCompare also lends itself to comparisons across TFs. Thus, we are applying BindCompare to analyze MLE and CLAMP's co-regulatory functionality. Because its only inputs are BED files, BindCompare can examine binding in organisms beyond Drosophila, including mice and humans.

A key focus of this platform is accessibility. The use of bioinformatics tools is often hindered by complex setup processes and package conflicts. BindCompare's Docker-mounted GUI interface allows for easy access to the method.

42 **The Effect of Long Non-coding RNA Expression on Vitamin E Concentration in Maize Grain** Morgan A Apolonio[1], Sam Herr[2], Michael A Gore[2] [1]University of California, Berkeley, [2]Cornell University

Tocochromanols are a group of antioxidants synthesized in plant tissues and have varying levels of vitamin E activity,

but tocochromanols with the highest vitamin E activity tend to occur at low levels in maize grain. In efforts to increase tocochromanol abundance in maize grain, Transcriptome-Wide Association Studies (TWAS) have been used to resolve quantitative trait loci (QTL) associated with grain tocochromanol concentrations to the gene level. While the causal loci underlying most of the large-effect QTL have been identified, relatively smaller effect QTL remain unresolved. Our past efforts have not explored the potential role of long non-coding RNAs (lncRNA), which are transcripts longer than 200 bp that do not encode a protein, in the genetic control of natural variation for tocochromanols in maize grain. Therefore, we hypothesize that lncRNAs could account for a portion of the unexplained genetic variation. We tested this hypothesis by conducting a new TWAS analysis in a maize association panel scored for lncRNA expression and grain tocochromanol levels. Nearly 20% of the identified lncRNAs were expressed in more than 10% of the individuals in the panel and were retained for the TWAS analysis. Of these, we found thirteen putative lncRNAs to be significantly associated with tocochromanols and of which four coincided with unresolved grain tocochromanol QTL identified in the maize nested association mapping panel. Our results highlight that including lncRNAs may be useful in resolving vitamin E-associated QTL in maize grain.

43 **Global proteome rewiring in times of desiccation and rehydration** Sheila Ferer, Nadine Zayyad, Hugo Tapia California State University Channel Islands

Most biological processes occur with water as a solvent, making desiccation a major stressor for living organisms. Anhydrobiotes—desiccation tolerant organisms—can overcome such adverse events by going into stasis and regain normal metabolic function once rehydrated. The subcellular localization of proteins can be affected by environmental perturbations. Global protein localization upon a detrimental stress as desiccation has never been evaluated. We will examine changes in the localization of proteins upon drying/rehydrating by utilizing the Yeast GFP-Fusion Collection. The *Saccharomyces cerevisiae* fusion library constitutes 75% of the yeast proteome, providing the opportunity to assay the physiological expression level and subcellular localization of 4,156 proteins. A preliminary pilot study on 20 differently localized GFP-tagged strains has revealed localization changes of the majority of the strains examined. Moving forward, automated-microscopy, alongside custom machine-learning algorithms will be used to capture and analyze images and cluster them based on similarity. Performing this for thousands of strains over different conditions will give us sufficient variability to generate a detailed roadmap of the yeast proteome and assess how it is rewired under desiccation stress. Proteins/protein families that change their localization pattern by a large degree are top candidates for factors implicated in (mal)adaptive responses to desiccation. These candidates will be subsequently characterized using functional assays (i.e., effect of knockdown on desiccation tolerance), as routinely performed in our laboratory. This undertaking will allow us to test how the proteome is rewired under desiccation and assess whether it constitutes an adaptive response priming the cell for recovery. Understanding anhydrobiosis and desiccation tolerance mechanisms is critical for tackling the climate crisis, space exploration, medical innovation, plant breeding, food spoilage, and gene banking.

44 **Using chromosome synthesis to study genetics and molecular evolution across yeast genera** Shawn H. Yang, Alessandro L.V. Coradini, Ian M. Ehrenreich Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California

The phenotypic diversity that evolution has generated is one of our best resources for understanding how organisms' genetic blueprints specify their phenotypes. However, as evolutionary distance between organisms increase, so do reproductive barriers and differences in genome structure, making it difficult, if not impossible, to perform conventional genetic analyses. We hypothesized that this impasse could be overcome through the synthesis of chromosomes possessing the gene content and organization of one genus but the DNA sequences of another. We are using the budding yeast model system to test this possibility. Specifically, we are attempting to make a *Saccharomyces cerevisiae* cell in which Chromosome I has a *S. cerevisiae* architecture but is composed of DNA sequences from *Kluyveromyces marxianus*. These two species diverged ~100 million years ago, show >50% nucleotide divergence, and possess completely different genome organization. As a pilot, we initially focused on ⅙th of the chromosome. We assembled this 1/6th chromosome segment in a single transformation of 27 partially overlapping, synthetic DNA fragments into an *S. cerevisiae* cell. RNA-seq experiments with this 1/6th chromosome segment confirmed that all 17 *K. marxianus* genes in the segment are transcribed and that expression of these *K. marxianus* genes does not cause any major changes in the *S. cerevisiae* transcriptome. We attempted to delete the corresponding 1/6th segment from the native *S. cerevisiae* genome but could not. Experiments with each of the individual genes revealed that a single gene–an essential component of the cleavage and polyadenylation complex (*PTA1*)–is the barrier to substituting the 1/6th chromosome segment between genera. We are now characterizing *PTA1* further, while also extending our work to the rest of the chromosome. This project could establish a new paradigm for how genetics can be experimentally studied across long evolutionary timescales that otherwise permit only descriptive, bioinformatic work.

45 **Investigating the effects of cannabidiol (CBD) on sleep, lifespan, and protein aggregation in a Drosophila melanogaster model of Parkinson's disease** Matthew J Irons, Kathryn A Jewett Biology, Juniata College

Our lab specializes in utilizing a *Drosophila melanogaster* Parkinson›s disease (PD) model featuring a genetic disruption in the human ortholog of the *GBA* gene, responsible for encoding the lipid-modifying enzyme glucocerebrosidase. Human *GBA* mutations are associated with an increased predisposition to PD, often precluded by initial symptoms such as sleep disturbance. With the relatively recent expansion of cannabidiol (CBD) sales within the United States, PD patients are turning to CBD with the hope of alleviating symptoms such as insomnia, pain, and dystonia. We seek to investigate the potential of CBD as a treatment in our PD *Drosophila* model.

Our total *GBA* deletion model (GBA^{Del}) flies are characterized by a shortened lifespan, impaired sleep, and increased protein aggregation. These three characteristics were measured after CBD exposure using STATA software, the Drosophila Activity Monitoring System (DAMs), and western blot. We have employed our GBA^{Del} with the DAMs to track sleep and activity patterns post-CBD ingestion. Leveraging the open-source ShinyR-DAM software, we observed a significant increase in both total and nighttime sleep after CBD exposure in the GBA^{Del} flies with significantly longer sleep bout length and no change in the number of sleep bouts. To further elucidate the effects of CBD with this PD model, we conducted a lifespan assay to assess the longevity of our model post-treatment. Through population sampling and subsequent western blot assays, we appraised the impact of CBD exposure on protein aggregation within our *Drosophila melanogaster* PD model.

46 **Adenylosuccinate alleviates mobility deficits associated with Adenylosuccinate Synthetase deficiency in *Caenorhabditis elegans*** Rishika R Patil, Latisha P Franklin, Wendy Hanna-Rose Biochemistry and Molecular Biology, The Pennsylvania State University

Inborn errors of purine metabolism have been linked to various neurological and muscular disorders that are under-reported and often go undiagnosed or misdiagnosed because their symptoms may often mimic other, more detectable, diseases. The links between purine metabolism and the mechanisms of these diseases remain understudied and effective therapies are unavailable due to our insufficient understanding of them. Therefore, these disorders are of major clinical significance and pose some of the most challenging enigmas in medical diagnosis and treatment. ADSSL1-Myopathy is an ultra-rare muscular disorder caused by a mutation in the purine nucleotide cycle (PNC) gene, ADSSL1. ADSSL1 codes for adenylosuccinate synthetase, an enzyme of the purine nucleotide cycle important for energy in the muscle. Some of the symptoms of the disorder include movement dysfunction, muscle weakness, lipid accumulation in the muscles, and the disruption of proper muscle structure. ADSSL1-Myopathy has been hard to characterize on a molecular level due to a lack of adequate models for its study. We are investigating the biological functions of ADSS using *C. elegans*. We have established that *adss-1* RNAi knockdown animals have slower crawling speed, reduced wavelength, reduced thrashing rate, and uncoordinated movement. They also exhibit an enlargement and accumulation of lipid droplets. We have probed the neuromuscular junction through paralysis assays using aldicarb and levamisole but found no obvious impairments. Additionally, we are currently examining muscle structure. We also hypothesize that the supplementation of PNC substrates will ameliorate phenotypes associated with the lowered expression of *adss-1* and can be used as a potential therapeutic strategy. We have found that the supplementation of adenylosuccinic acid (ASA) rescues movement phenotypes of speed and wavelength and are continuing to analyze other parameters of movement. This supports the use of other PNC substrates as a therapeutic strategy for the disorder.

47 **Tapping into worm conversations: transfer of memory from one *Caenorhabditis elegans* to another and investigating its underlying molecular mechanism** Monmita Bhar[1], Hari Pradeep Narayanan[2], Shrinithi Natarajan[3], Tanumoy Nandi[4], Kamal Kishore[4], Kavita Babu[4] [1]Centre for Neuroscience, Indian Institute of Science, [2]Neurobiology, University of Konstanz, [3]Computer Science and Engineering, PES University, [4]Centre for neuroscience, Indian Institute of Science

Native preference in organisms can be modified through experience. *Caenorhabditis elegans* has been proven to be an extremely important model system to study behavioural plasticity. In this study, we looked at long-term associative memory (LTAM) in *C. elegans* using two cues – the native chemoattractant isoamyl alcohol (IAA) along with heat pulses. After this training paradigm, worms are incubated at ambient temperatures (22°C) in the incubator for the next 20-24 hours. LTAM is seen in the form of loss of attraction towards IAA due to this aversive learning paradigm. Here we report that worms lose this LTAM when removed from the plates they are trained on. This suggests that during training, *C. elegans* release some factors onto the plate that act as signaling molecules for maintaining the LTAM. Hence, removal of worms from these plates causes them to lose their memory despite undergoing aversive training.

Naïve worms that have not undergone the training can also take up these factors if exposed to the trained plates. This leads to LTAM formation in naïve worms, specific to IAA, causing them to lose their attraction towards this chemoattractant. The same is seen for memory-defective mutant worms, where they can take up these factors released by wild-type trained worms, while themselves not being able to release them during training.

To investigate the underlying molecular mechanism, we have performed RNA sequencing to look at differential gene

expression in worms that have LTAM versus worms that lose it. We have identified some candidate genes that may be responsible for this phenomenon. Additionally, to identify the externally released factors, we have performed liquid chromatography mass spectrometry (LC-MS) from the plates worms were trained on.

Here, we propose a mechanism by which *C. elegans* due to aversive training, release some factors onto the plate, which may be in a compartmentalized fashion through environmentally released extracellular vesicles (EVs), which can further be taken up by any worm (naïve untrained or memory-defective mutants) and get processed downstream, leading to similar behavioural phenotype in these untrained worms. Hence, in this study, we report that memory (LTAM) can be transferred from a trained worm to an untrained worm, opening a new avenue of communication and signaling with respect to long-term associative memory formation in *C. elegans*.

48 **TOP-2 is differentially required for meiotic chromosome morphology in spermatogenesis and oogenesis** Christine K Rourke[1], Lauren Salvitti[2], Gabriella Gassaway[3], Aimee Jaramillo-Lambert[1] [1]Biological Sciences, University of Delaware, [2]Charles River Laboratory, [3]Rutgers University

The specialized cell division of meiosis results in the production of haploid gametes from diploid gamete precursor cells. The success of meiosis I is dependent on the proper pairing of homologous chromosomes, synapsis, and recombination. Failure to complete these steps properly results in gamete aneuploidy, which is the leading cause of infertility, progeny inviability, and birth defects. Many meiotic events are sex-specific including chromosome structure. In particular, the degree of chromosome compaction in spermatogenesis is much greater than in oogenesis. Differential chromosome compaction is coordinated by several proteins including sperm-specific histone variants and post-translational modifications, condensins, cohesins, and DNA topoisomerases. Previously, we identified a loss-of-function mutation of *C. elegans* Topoisomerase II, *top-2(it7*). In *top-2(it7)* homologous chromosomes fail to segregate resulting in aneuploid sperm and embryonic lethality after fertilization. Recently, we found that *top-2* plays sex-specific roles in the localization of meiotic chromosome structural components and on chromosome structure during late meiotic prophase. During spermatogenesis, late prophase chromosomes are significantly compromised in their ability to condense and individualize after loss of *top-2* function. During oogenesis, chromosome structure appears to be unaffected in *top-2(it7*), however, diakinesis bivalent length is elongated. These sex-specific differences are likely due to differences in the temporal regulation of late meiotic prophase events. As to*p-2(it7)* changes the chromosome morphology of meiotic chromosomes in both spermatogenesis and oogenesis, we asked if *top-2(it7)* changes the recombination landscape. We examined meiotic double-strand break repair by the assembly and disassembly of RAD-51 foci during spermatogenesis and oogenesis. We found fewer RAD-51 foci in the transition zone through late pachytene in *top-2(it7)* spermatogenic and oogeneic germlines. Even though fewer RAD-51 foci are observed, crossover formation is not perturbed in *top-2(it7)* spermatogenesis nor oogenesis as five and six GFP::COSA-1 foci, respectively, were found per nucleus in the mutant germlines. However, *top-2(it7)* appears to alter the position of crossovers in spermatogenic germlines. Currently, we are further probing the role of TOP-2 through a combination of FISH oligopaint and super-resolution microscopy to analyze chromosome structure.

49 **How does a complex genomic rearrangement affect gene expression?: A research project for undergrads in a classroom setting** Tatiana Maroilley[1,2], Victoria Rodrigues Alves Barbosa[1,2], Diogo Marques[3], Catherine Diao[1,3], David Anderson[1,4,5], Maja Tarailo-Graovac[1,3,5] [1]Biochemistry and Molecular Biology, Cumming School of Medicine, University of Calgary, [2]Medical Genetics, Alberta Children Hospital Research Institute, niversity of Calgary, [3]Medical Genetics, Alberta Children Hospital Research Institute, University of Calgary, [4]Alberta Children Hospital Research Institute, University of Calgary, [5]co-last authors

Structural variants (SVs) are independent large genomic rearrangements (>50 bp): copy number variants (deletions, duplications), inversions, and translocations. Cascades of catastrophic events can create complex genomic rearrangements (CGRs) or chromoanagenesis, involving multiple SVs. Frequent in tumor cell genomes, they can also cause rare genetic diseases. But they are understudied in clinical diagnosis as their detection with low-resolution genetic techniques (FISH, arrays) prevents a complete molecular characterization and interpretation of pathogenicity.

Caenorhabditis elegans balancer strains are well described phenotypically, and their genomes carry SVs/CGRs. We have recently used short-read whole genome sequencing to characterize these events at a base-pair resolution (Maroilley et al. 2021, 2023). In addition, we have reported all SVs/CGRs detectable in the genomes of the strains, beyond the balanced regions, publishing then the first catalog of SVs/CGRs (on average >5 per strain, and overall >100). Here, we explore the effects each SV/CGR has on gene expression by sequencing bulk RNAs using short reads.

We have implemented our research project with a class of undergraduate students over a semester as an innovative teaching tool to introduce research in Bioinformatics. Our curriculum was developed as a CURE, a Course-based Undergraduate

Research Experience for Bioinformatics program students (Bachelor of Health Sciences, Cumming School of Medicine, University of Calgary). We first trained the students in data manipulation and WGS data analysis to detect SVs/CGRs. Then, they analyzed the transcriptome data of the same strains to scrutinize the potential effect of SVs and CGRs on gene expression and splicing. We organized an in-person conference for students to present their findings to a large audience.

We uncovered that the reciprocal translocation *eT1(III;V)* affects two genes: it prevents the transcription of *unc-36* by RNA truncation, explaining the uncoordinated phenotype of the carriers. In addition, our data shows that *eT1* activates the transcription of an understudied gene *H14N18.2*, which is not expressed in control.

Our project combines teaching and advanced Bioinformatics in a research project with original multi-omics data as way to offer a research experience to undergraduate students. Ultimately, our analyses should reveal new genome-transcriptome-phenotype associations and possibly uncover new genes, gene function, or transcripts.

50 **A LIN-42-Casein kinase 1δ interaction is necessary for C. elegans developmental timing** Rebecca K Spangler[1], Guinevere Ashley[2], Kathrin Braun[3], James M Ragle[2], Carrie L Partch[1], Helge Grosshans[3,4], Jordan D Ward[2] [1]Chemistry and Biochemistry, UC Santa Cruz, [2]Molecular, Cell, and Developmental Biology, UC Santa Cruz, [3]Friedrich Miescher Institute for Biomedical Research, [4]University of Basel

All living organisms use timing mechanisms to coordinate developmental, cellular, and physiological processes over time. Two independent but interconnected biological timers drive progression through C. elegans development. The heterochronic pathway is a linear timer that controls the serial progression of stage-specific cellular events. A cyclical molting timer coordinates apical extracellular matrix regeneration and shedding of the old cuticle. Failure to coordinate these two timers leads to animal death, but how these timers are coordinated is unclear. LIN-42, the C. elegans homolog to the mammalian circadian clock protein PERIOD2 (PER2), has been established as a key component of both timers. To gain insight into how LIN-42 coordinates developmental timing, we looked for novel interactors through co-IP mass-spectrometry and found an interaction with KIN-20, the C. elegans homolog of mammalian Casein Kinase 1δ (CK1δ). In mammals, circadian clocks are controlled by a transcription-translation feedback loop of PER2 expression and degradation. The stability of PER2 is largely determined through its interaction with CK1δ, a kinase that binds to and phosphorylates PER2 at one of two regions leading to either stabilization or degradation of PER2. Sequence analysis indicates two highly conserved kinase-binding motifs, and our in vitro pull-down assays show that these domains are conserved enough in LIN-42 to maintain interactions with CK1δ suggesting that a common mechanism may anchor the kinase to LIN-42. The LIN-42 C-terminus is phosphorylated by CK1δ to a similar degree and within the same timeframe as CK1δ phosphorylation of a PER2 substrate. Deleting the conserved kinase binding motifs abolished the interaction with CK1δ and reduced phosphorylation of the LIN-42 C-terminus compared to wildtype. When we engineered the equivalent mutations into the lin-42 endogenous locus, we observed profound developmental delay as well as extended and asynchronous molts. Surprisingly, deletion of the conserved PAS domains which had been previously implicated in the heterochronic pathway, only produced mild heterochronic defects. In contrast, deletion of the unstructured C-terminus of the protein produced much stronger heterochronic defects. Together, these data suggest that PER2/LIN-42 phosphorylation by CK1δ kinases is evolutionarily conserved and that this mechanism may be employed by the molting oscillator to coordinate developmental timing.

51 **The molecular atlas of *C. elegans* glia across sexes reveals sexually dimorphic and heterogeneous glia** Maria D Purice[1], Elgene J. A. Quitevis[1], Richard S Manning[1], Liza Severs[1], Nina-Tuyen Tran[1], Manu Setty[1,2], Aakanksha Singhvi[1,3] [1]Basic Sciences, Fred Hutchinson Cancer Center, [2]Herbold Computational Biology Program, Public Health Sciences, Fred Hutchinson Cancer Center, [3]Department of Biological Structure, University of Washington School of Medicine

Interactions between neurons and glia are essential for nervous system function. Recent studies suggest glia heterogeneity and their functions differ across brain regions and disease. However, the extent of glia molecular diversity across the entire nervous system, across sexes, or how glia molecular diversity impacts circuit function, remains largely unknown. *C. elegans* has an invariant nervous system with the first mapped connectome of a multicellular organism across both sexes. Here we present single nuclear RNAseq evaluation of glia across the entire nervous system of adult *C. elegans* in both sexes, complementing previous single-cell analysis of its neurons and connectome. Our data identify both sex-shared and sex-specific glia. We have identified glia-specific markers of which we have validated *in vivo* using transcriptional reporters. Also, iterative computational and machine learning models reveal glial subclasses, of which we have also identified subtype-specific markers for and validated *in vivo*. Overall, this molecular atlas reveals detailed insights into glial heterogeneity and sex dimorphism in the adult *C. elegans* nervous system. Our data is made available as a searchable atlas at wormglia.org.

52 **Genetic Integration of Multi-Omics Data: Realizing the Promise of Genetical Genomics** Gary Churchill The Jackson Lab

It is now more than 20 years since Jansen and Nap (2001) proposed genetical genomics, a merger of genomics and genetics, as a new approach for unraveling of metabolic, regulatory and development pathways. In the intervening time, advances in our ability to quantify the molecular components of biological samples combined with the development of powerful model organism genetic resources have changed genetical genomics from aspiration to routine practice. This talk will briefly review some of the key advances that made this possible, and present examples of multi-omics integration that leverage genetic variation to establish causal linkages across chromatin structure, gene expression, protein abundance, post-translational modifications, metabolites, and cell-based and whole organism phenotypes.

53 **Effects of sex-linked variation and paternal age on recombination rate in house mice** Andrew P Morgan Department of Medicine, University of North Carolina

Recombination is the defining feature of sexual reproduction. In mammalian meiosis, the formation of crossovers between homologous chromosomes is critical for the faithful segregation of chromosomes to gametes, and thus for fertility. The rate and distribution of recombination across chromosomes modulate the impact of both genetic drift and natural selection on patterns of genetic diversity in populations. Characterizing the genetic and life-history factors that control the rate of recombination is therefore fundamentally important for understanding the evolution of mammalian genomes.

To this end we analyzed genotypes of 503 progeny from reciprocal F1 males between inbred strains representing the three major subspecies of house mice (Mus musculus), a well-established model system for the study of recombination and speciation. Recasting the experiment as a classical diallel design, we apply a hierarchical Bayesian model to estimate the contribution of additive, dominance and parent-of-origin effects to variation in male recombination rate. We show that a paternally-inherited factor from the *M. m. musculus* subspecies increases the global recombination rate by 0.5 crossovers per meiosis (4.3%). Furthermore, aged males transmit an average of 0.6 more crossovers per meiosis (5.1%) than young males independent of the genetic background. Our design also permits direct estimation of the strength of crossover interference. We implement a well-known model of interference in a hierarchical Bayesian framework and show that crossover interference in the male germline weakens with increasing age at paternity; the effect is variable across backgrounds. Our results highlight a previously unappreciated role of the Y chromosome in control of recombination in hybrid males and hint at further links between recombination, the sex chromosomes and speciation in mice.

54 ***De novo* structural variants on the sex chromosomes of CC029/Unc mice cause meiotic segregation errors in F1 hybrid males and lead to a high rate of sex chromosome aneuploidy** Rachel M Lynch[1,2,3], Pablo Hock[1], Matthew W. Blanchard[1,4], Caroline E. Murphy[1], Michelle E Allen[1,2], Timothy A Bell[1], Madison A Drushal[1,2], Martin T Ferris[1,2], Richard Austin Hodges[1,2], Colton L Linnertz[1], Md Taksir Hasan Majumder[5], Darla R Miller[1,2], John Sebastian Sigmon[4,5], Ginger D Shaw[1], Mark J Zylka[6,7], Fernando Pardo-Manuel de Villena[1,2,4,8] [1]Genetics, University of North Carolina at Chapel Hill, [2]Systems Genetics Core Facility, University of North Carolina at Chapel Hill, [3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, [4]MMRRC at UNC, University of North Carolina at Chapel Hill, [5]Computer Science, University of North Carolina at Chapel Hill, [6]Cell Biology and Physiology, University of North Carolina at Chapel Hill, [7]UNC Neuroscience Center, University of North Carolina at Chapel Hill, [8]Lineberger CLineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

XO and XXY sex chromosome aneuploidies (SCA) are the most common types of viable aneuploidies in both mice and humans. In mice, a significant excess in SCA has been reported in the progeny of (CC029/Unc x CC030/GeniUnc)F1 males. Here we characterize the rate, parental and meiotic origin, and genetic basis of this phenomena. We analyzed sex chromosome segregation in 11 crosses that involve CC029 or its F1 derivatives. Eight are 4-way reciprocal F2 crosses between CC029 and other CC strains. Two are outcrosses between B6 females and reciprocal (CC029 x CC030)F1 males, and the last is the CC029 colony itself. We show that increases in SCA rates are restricted to the progeny of F1 males leading to a ~20-fold increase in SCA over baseline (~0.35 +/- 0.04%). However, the two types of reciprocal F1 males differ significantly in the rate and type of SCA observed. F1 males carrying an X chromosome derived from CC029 have the highest SCA rate (9.32 +/- 1.32%) and produce similar numbers of XO females and XXY males. F1 males carrying a Y Chromosome derived from CC029 have a lower SCA rate (2.27 +/- 0.86%) and only generate XO females. Analysis of the XXY progeny of the outcross unambiguously points to errors in chromosome segregation in meiosis I as the origin of SCA. This conclusion is consistent with the data of all other crosses. XO females could also be explained by meiosis II errors in male meiosis. To identify candidate mutations, we scanned the genome sequence of the CC029 sex chromosomes for *de novo* mutations, focusing on structural variants that may impair pairing, recombination and/or segregation of the sex chromosomes. There is a large known Y Chromosome deletion. In addition, we identified a 520 Kb interval of the distal X Chromosome that is translocated to the Y Chromosome. Recombination analysis suggests that this region is excluded from the pseudoautosomal region in males and reduces recombination in females. We will present a unified chromosomal model that is consistent with the observed data. In conclusion, CC029 is a new model for studying the mechanics of sex chromosome segregation in the male germline. It also supports a design to

systematically study the phenotypic consequences of SCA and X chromosome inactivation in genetically variable mice. Finally, researchers using CC029 in crosses should account for the presence of a significant number of aneuploids.

55 **The X and Y of sex disparities in cancer: sex chromosomes contribute to the male bias in melanoma progression** Nora Engel, Gillian McGuire, Daniel Deegan, Kiera Patton Coriell Institute for Medical Research

Melanoma is a quintessential example of a non-reproductive cancer with differences between males and females in incidence, progression, and response to therapy. Long-established epidemiological data show higher incidence and worse outcomes for male melanoma patients. These imbalances have not been explained and suggest that there are protective and susceptibility factors distinct between males and females that could be targeted for better outcomes. Yet currently, standard-of-care therapies for melanoma, or for that matter, any non-reproductive cancer, are delivered without consideration of the biological sex of the patient. Our goal is to elucidate the mechanisms underlying the sex disparities in melanoma and lay the groundwork for future development of sex-aware therapeutic strategies.

The two main factors that contribute to sex differences are the sex chromosome composition and sex hormones, collectively shaping a sex-specific transcriptomic and epigenetic landscape in normal somatic cells as well as in cancers. **Our hypothesis is that sex differences in melanoma result from these two factors via mechanisms that are intrinsic to the tumor cells and mechanisms related to the host.**

Because sex chromosome and sex hormone effects are coupled and covary with each other, we use the Four Core Genotypes model to disentangle these factors by producing XX and XY males and XX and XY females . In this mouse model, the sex chromosome composition is independent of the gonadal sex, allowing us to identify independent and joint effects of each factor. We challenged the FCG mice with male and female melanoma cell lines and compared melanoma growth as well as the transcriptomes and epigenomes of the resulting tumors. The design allowed us to identify sex differences intrinsic to the melanoma cells injected in addition to host effects dependent on the sex chromosome composition and gonad type. With a focus on transcriptional and epigenetic sex-biased genes, we genetically manipulated candidate factors and validated important regulatory nodes that underlie the sex differences in melanoma progression. Our studies establish a compelling framework to identify sources of sex biases in cancer that have traditionally been disregarded.

57 **Cas13d Unveils the Essential Role of Cell-Specific Maternal RNA Degradation in Zebrafish Development** Gopal Kushawah[1], Ariel Bazzini[2] [1]Ariel Bazzini lab, Stowers institute for medical research, [2]Stowers Institute for Medical Research

Maternal transcripts are essential in early embryonic development, providing a foundation for zygotic genome activation. They undergo time-dependent degradation and shift the control from maternal to zygotic transcripts (MZT). Our study focuses on the often-overlooked aspect of spatial regulation of maternal RNA degradation, hypothesizing that both spatial and temporal control are essential for the development of complex, multicellular embryo. Exploring the regulation of dynamic spatiotemporal changes in maternal mRNA degradation, stabilization or localization in a cell-specific manner and their impact on early embryonic development, continues to be a subject of intense investigation. To unravel this, we initially identify and selected the spatiotemporal maternal RNAs through a combination of RNA-seq, Slam-seq, and Seurat- digital RNA *in situ* predication tool. We further validated their degradation patterns by RNA *in situ* hybridization assays in zebrafish embryos. To gain deeper insights into their functional importance, we attempted to deplete maternal RNAs by creating maternal-zygotic Cas9 mutants. However, this approach led to embryonic lethality for few candidates, posing a challenge in validate our hypothesis. Subsequently, we turned to CRISPR-Cas13d to specifically deplete these spatio-temporal RNAs, which led to specific spatial context-specific phenotypes during embryonic development. Notably, the F0 CRISPR-Cas9 mutants for corresponding genes confirmed that these phenotypes were independent of any interference from zygotic genes. Overexpression experiments provided additional confirmation of the significance of the observed spatiotemporal RNA gradient, as the developing embryos could not tolerate the presence of extra copies of these RNAs. Additionally, our RNA reporter assays identified specific cis-elements within maternal RNAs responsible for the spatial-temporal RNA degradation during embryogenesis. Our future objective is to investigate the evolutionary functional significance of these spatiotemporal maternal RNAs during development and diseases.

58 **Characterizing a tissue-biased *tp53*-mediated DNA damage response in early zebrafish development** Sean Lee[1], Eric Upton[1], Kathryn Berg[2], Alexander F Schier[2], Jeffrey A Farrell[1] [1]NICHD, NIH, [2]Molecular and Cellular Biology, Harvard University

DNA damage is a normal occurrence during embryogenesis and a proper DNA damage response (DDR) is essential for maintaining genome integrity and healthy development. A standard DDR is characterized by the activation of a network of repair mechanisms, cell cycle checkpoints, and programmed cell death signals. Previous single-cell RNAseq (scRNAseq) data revealed that zebrafish embryos express a modified DDR module hallmarked by the simultaneous transcription of many developmental regulatory genes (e.g., *aplnrb* and *igf2a*) alongside keystone stress response genes (e.g., *tp53*, *cdkn1a*,

and *gadd45aa*). Single whole embryo bulk RNAseq data suggests that the module genetically regulated by *tp53* and temporally restricted to the blastula stage, prior to gastrulation. Reanalysis of the scRNAseq data highlights a tissue bias: module-expressing cells are more prevalent than expected in the non-neural ectoderm and less prevalent in the axial mesoderm. *In situ* hybridization chain reaction (HCR) in wild-type embryos supports this tissue bias. HCR in the Nodal signaling mutant, *MZoep*, and BMP signaling mutant, *swr*, indicates that this bias is linked to dorsal-ventral patterning signals. Together, our results demonstrate stage-specific activation of a non-neural ectoderm-biased DDR module. My future experiments will investigate: 1) whether non-neural ectoderm identity mediates this bias, 2) whether the bias results from differences in the amount of damage or the response to damage across different cell types, and 3) the mechanisms that mediate the decreased module expression in the axial mesoderm. Funding: NIH Intramural ZIAHD008997 to JAF.

59 **A New Mechanism of Dorsal Axial Organizer Repression by Integrator Complex Subunit 6** William D. Jones, Mary C. Mullins Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine

The vertebrate body axis is established by the dorsal organizer, an embryonic signaling center that promotes dorsal fates. A unique zebrafish loss-of-function mutant from our laboratory, called *ints6*^{p18ahub}, provides new insights into how the organizer is repressed to specify the proper balance of dorsoventral fates. In this maternal-effect mutant, the progeny of homozygous *ints6*^{p18ahub} mutant females (M-*ints6*^{p18ahub} embryos) display an expanded organizer and multiple body axes. The mutated gene, *integrator complex subunit 6* (*ints6*), encodes a subunit of the Integrator complex, which has not previously been implicated in embryonic patterning. Integrator was first discovered as a spliceosomal small nuclear RNA maturation factor, but it can also regulate gene expression via its endonuclease and phosphatase modules. In this project, I aim to uncover the mechanism by which Ints6 represses the organizer. I have found that *ints6*^{p18ahub} is a hypomorphic, temperature-sensitive (ts) allele, and that maternal-effect nonsense alleles of *ints6* cause an earlier mid-blastula arrest phenotype. To test the timing of Ints6 function, I performed temperature-shifts at different stages using the *ints6*^{p18ahub} ts allele. I found that Ints6 first functions to repress the organizer at a mid-blastula stage, shortly after zygotic transcription begins. Next, I tested the spatial function of Ints6, to put Ints6 into the context of other organizer repressors that act either dorsally or ventrolaterally during zebrafish dorsoventral patterning. Using regional expression of wild-type Ints6 in M-*ints6*^{p18ahub} embryos, I found that Ints6 expressed ventrolaterally can rescue development, whereas Ints6 expressed dorsally in the organizer does not. This indicates that Ints6 acts ventrolaterally, not dorsally, to repress the organizer. Finally, I performed RNA-seq in *ints6* maternal mutant and control embryos and found thousands of differentially expressed genes (DEGs). Surprisingly, over 90% of DEGs were upregulated in *ints6* maternal mutant embryos during the mid-blastula period, supporting the hypothesis that Ints6 represses early zygotic expression of many genes. In general, Integrator can repress gene expression by cleaving nascent RNAs with its endonuclease module or by recruiting Protein Phosphatase 2A to dephosphorylate the C-terminal domain of RNA polymerase II. I will report on candidate dorsalizing genes that I am testing as targets of Ints6 repression to restrict the dorsal organizer.

60 **BMP receptor trafficking and specialization in signal transduction during embryonic patterning in the zebrafish** Jeet Patel, Benjamin Tajer, Mary C Mullins Cell and Developmental Biology, University of Pennsylvania

Bone morphogenetic protein (BMP) signaling is a major driver of developmental processes, including bone development, organ system formation, and patterning of the dorsoventral (DV) axis, neural tube, and limbs. The role of BMP signaling in DV patterning is conserved from insects through humans, one interesting feature of which is signaling by a BMP heterodimer. In zebrafish, this Bmp2-Bmp7 heterodimer signals through a heteromeric BMP receptor complex of the Type I receptors Acvr1l and Bmpr1, along with two Type II Acvr2 receptors. The Mullins lab recently showed that, while both Type I receptors are required for embryonic patterning, the kinase of only Acvr1l is active in phosphorylating Smad1/5 in the zebrafish, while Bmpr1a kinase activity is dispensable. While prior paradigms postulated that both Type I receptors activate downstream Smads, the specialized use of a single kinase when two are available has also been observed in mouse granulosa cell culture. The biological basis for specialized receptor kinase functions presents a range of possible mechanisms by which different receptors could be leveraged to regulate signaling in diverse contexts. Using live-imaging approaches, I have found that Acvr1l is trafficked intracellularly via endocytosis, while Bmpr1a is primarily localized to the membrane and rarely trafficked into endosomes. As Acvr1l is the Type I receptor with required kinase activity, its receptor trafficking may facilitate BMP signal transduction. Indeed, Acvr1l and Acvr2 are frequently found in the same endosomes. Trafficking of these receptors together is consistent with a model in which Acvr2 can phosphorylate Acvr1l to activate Smad5 in the gastrula. Overexpression of both Acvr1l and Acvr2 leads to an increase in Acvr1l positive endosomes and embryonic ventralization, which is not seen with either receptor alone. Interestingly, more Acvr1l endosomes are observed with constitutively active Acvr1l, which obligately phosphorylates Smad5 to ventralize the embryo, suggesting a link between receptor trafficking and signal transduction. Using mutants for extracellular BMP regulators, BMP receptors, and Smad5, I will further interrogate the requirements for endocytosis of Acvr1l in signal transduction and embryonic patterning, defining the cell biological mechanisms of BMP signaling regulation in vertebrate development.

61 **Single-cell temporal dynamics reveals the relative contributions of transcription and degradation to cell-type specific gene expression in zebrafish embryos** Lior Fishman[1], Gal Nechooshtan[1], Avani Modak[2], Florian Erhard[3], Aviv Regev[4], Jeffrey Farrell[2], Michal Rabani[1] [1]Hebrew University of Jerusalem, [2]National Institute of Child Health and Human Development, [3]University of Wurzburg, [4]MIT

During embryonic development, pluripotent cells assume specialized identities by adopting particular gene expression profiles. However, systematically dissecting the underlying regulation of mRNA transcription and degradation remains a challenge, especially within whole embryos with diverse cellular identities. Here, we collect temporal cellular transcriptomes of zebrafish embryos, and decompose them into their newly-transcribed (zygotic) and pre-existing (maternal) mRNA components by combining single-cell RNA-Seq and metabolic labeling. We introduce kinetic models capable of quantifying regulatory rates of mRNA transcription and degradation within individual cell types during their specification. These reveal different regulatory rates between thousands of genes, and sometimes between cell types, that shape spatio-temporal expression patterns. Transcription drives most cell-type restricted gene expression. However, selective retention of maternal transcripts helps to define the gene expression profiles of germ cells and enveloping layer cells, two of the earliest specified cell-types. Coordination between transcription and degradation restricts expression of maternal-zygotic genes to specific cell types or times, and allows the emergence of spatio-temporal patterns when overall mRNA levels are held relatively constant. Sequence-based analysis links differences in degradation to specific sequence motifs. Our study reveals mRNA transcription and degradation events that control embryonic gene expression, and provides a quantitative approach to study mRNA regulation during a dynamic spatio-temporal response.

62 **Defining the Molecular and Neural Roles of the Understudied Kinase *vrk2* in Zebrafish** Jaqueline Martinez[1,2], Vaishnavi Balaji[1,2], Claire Conklin[2], Summer Thyme[1,2,3] [1]BMB, UMass Chan Medical School, [2]University of Alabama at Birmingham, [3]Harvard University

Genetic variation adjacent to vaccinia-related kinase 2 (VRK2) is associated with schizophrenia, bipolar disorder, sleep, and major depression. Preliminary work using phospho-Erk brain activity mapping and behavior analysis revealed altered sleep and reduced brain activity, particularly in the rostral hypothalamus that is involved in sleep. We aim to define the molecular, cellular, developmental, and behavioral processes regulated by *VRK2*. Repeating the behavioral experiments confirmed that these loss-of-function mutants had reduced baseline movement rates during the day but similar nighttime activity to control siblings. This behavioral difference was not retained in the presence of constant light or constant dark, indicating a role for light cycling in the phenotype. We hypothesized the cause for the behavioral disturbances in the mutant zebrafish was a lack of phosphorylation of its targets. To discover these targets, we are using an analog-sensitive kinase approach and biotin labeling pull-down experiment to label nearby interacting partners of *vrk2* that could be its substrates. As a first step in the analog-sensitive kinase method, we have generated versions of *vrk2* that have mutations in the gatekeeper residue to accept ATP analogs. Testing these mutated *vrk2* proteins by injection of mRNA in zebrafish embryos and *in vitro* kinase assays indicates that they retain functionality. We will expose adult zebrafish brain lysate to purified mutant kinases to label substrates and identify them with mass spectrometry. RNA-seq analysis of larval zebrafish heads from the loss-of-function line revealed changes to the levels of genes involved in the brain, such as an acetylcholine transporter, indicating that absent Vrk2 target phosphorylation likely has downstream impacts on neuron development or function. By defining the functions of genes such as VRK2 that are associated with an elevated risk of neuropsychiatric disorders, we aim to establish a foundational understanding of their origins, ultimately leading to the development of novel diagnostics and therapies.

63 **Fate switching of melanophores to leucophores requires Agouti and BMP** Dylan Huang, David Parichy UVA

Plasticity in cell fate allows mature cells to alter their identities and functions and has been most often studied in the context of tissue and organ regeneration. Yet emerging single cell and fate mapping technologies suggest such plasticity is more common during development than previously appreciated. Generalizable features of cell fate plasticity and its specific underlying mechanisms are of fundamental biological interest whereas the salient principles and specific genes and pathways involved have obvious therapeutic potential. Pigment cells in the dorsal fin of zebrafish offer a useful paradigm to investigate cell fate plasticity. In this system black, melanin-containing melanophores transdifferentiate to become white, guanine crystal-containing leucophores. Here we show this transition is regulated by signaling and crosstalk involving Agouti/Melanocortin and Bone morphogenetic protein (BMP) pathways. Leucophores develop from melanophores in the dorsal fin tip where Agouti signaling protein 1 (Asip1) serves as an environmental cue that represses differentiation of new melanophores, while Asip2b serves as a complementary factor that promotes the transition of previously differentiated melanophores into leucophores. Asip2b further promotes the competence of these cells to respond to local BMP signals that are themselves required for transdifferentiation. Our analyses indicate that BMP induces expression of *foxd3*, encoding a bifunctional forkhead family transcription factor that upregulates the purine synthesis gene *purine nucleoside phosphorylase 4a*, which is required to produce white guanine crystals. Taken together, our several approaches reveal an unanticipated signaling network that drives

a pigment cell fate transition in zebrafish, new genes involved in pigment cell differentiation, and new functional contexts for genes previously studied in other aspects of pigment cell development.

64 **Post-transcriptional regulation of gene expression in time and space** Howard Lipshitz University of Toronto

Early animal embryos represent an ideal system in which to study post-transcriptional regulation of gene expression because their genomes are transcriptionally silent and developmental processes are controlled by maternally provided RNAs and proteins. During a process known as the maternal-to-zygotic transition (MZT) a subset of maternal products is eliminated, permitting transcriptional activation of the zygotic genome. RNA-binding proteins (RBPs) play an essential role in post-transcriptional regulation during the MZT. Notably, the Drosophila Smaug RBP is required for repression and degradation of maternal transcripts during the MZT, working together with its partners: Cup, Trailer hitch (TRAL) and ME31B for translational repression; the CCR4-NOT deadenylase complex for transcript degradation. The timing of expression of Smaug and its partners is precisely regulated post-transcriptionally and post-translationally. At the beginning of the MZT, the Pan gu kinase triggers translation of the maternally provided *smaug* mRNA, leading to rapid accumulation of Smaug protein. Subsequently, two E3 ubiquitin ligase complexes – CTLH and SCF – trigger the sequential clearance from the bulk cytoplasm of Cup-TRAL-ME31B and of Smaug, respectively. SCF function and, thus, Smaug degradation, is timed by zygotic synthesis of a novel F-Box protein, Bard. Deletion of the C-terminal region of Smaug abrogates interaction with and clearance by SCF, resulting in persistence of Smaug beyond the end of the MZT and consequent downregulation of zygotically synthesized mRNAs that carry Smaug-binding sites. In contrast to its clearance from the bulk cytoplasm, Smaug protein is transported into and persists in the germ plasm, where it binds to and represses translation of several mRNAs, including *oskar* and *Bruno 1*. Mutation of either Smaug or cis-acting Smaug-binding sites in these targets results in synthesis of excess germ plasm and budding of supernumerary primordial germ cells from the posterior pole of embryos. Temporal and spatial regulation of Smaug expression and function exemplify the crucial role that post-transcriptional processes play during early embryogenesis.

65 **Examining the molecular mechanisms of fat-to-ovary communication in *Drosophila melanogaster*** Alissa R. Armstrong, Tancia R Bradshaw University of South Carolina

Organismal nutritional status influences reproductive output by impacting gamete production. The energy-intensive process of oogenesis requires coordinated nutritional responses between multiple organs, yet not enough is known about how inter-organ communication modulates tissue responses to dynamic nutritional input. In *Drosophila melanogaster* females, the stem cell-supported ovary sustains robust reproductive capacity and is sensitive to dietary changes. Female flies fed suboptimal diets, e.g. protein poor, high sugar, or high fat, display significantly reduced egg production rates. This response to diet is mediated by highly conserved nutrient-sensing pathways that function tissue autonomously, within the ovary, as well as non-autonomously, within the adipose tissue. We leverage the power of *Drosophila* genetics to uncover the cellular and molecular mechanisms that underlie fat-to-ovary communication about diet. We have shown that amino acid sensing, via the amino acid response (AAR) pathway and mTOR, and insulin/insulin-like growth factor signaling (IIS) within adipocytes controls multiple steps of oogenesis, including germline stem cell maintenance, germline survival, and ovulation, in a complex manner. We find that the AAR pathway suppresses translation of unknown adipocyte factors that promote germline stem cell maintenance while mTOR supports translation of unknown adipocyte factors that promote ovulation. In addition to our previous work showing that distinct signaling axes downstream of insulin receptor/PI3 kinase activity control different aspects of oogenesis, we have uncovered a role for activity of the Ras/MAPK signaling axis in mediating fat-to-ovary communication. Our data suggests the Ras/MAPK signaling in adipocytes controls germline cyst survival and ovulation. Ultimately, we aim to identify adipocyte factors downstream of IIS, AAR pathway, and mTOR that modulate *Drosophila* oocyte development. This work will illuminate how inter-organ communication coordinates organismal nutritional status with oocyte production.

66 **Building Tissue Specific Centrosomes** Nasser Rusan NHLBI, NIH

The Rusan lab has a longstanding interest in the mitotic spindle and the centrosome. The biology of these two microtubule-based molecular machines is highly intertwined, as centrosomes nucleate and organize the microtubules required for spindle assembly in most animal cells. To study these machines, we employ a wide range of techniques, including genetics, micro-computed tomography (uCT) scanning, yeast 2-hybrid screening, super-resolution microscopy, and live-cell imaging. Most importantly, we utilize the model organism *Drosophila* in combination with a powerful method of generating novel separation-of-function alleles. Our work has revealed fascinating tissue-specific centrosome functions in the contexts of asymmetric cell division, brain development, early embryo development, and spermatogenesis. Understanding this functional diversity of centrosomes has provided insight into centrosome dysregulation, which has been linked to many human diseases such as microcephaly and infertility. I look forward to sharing my lab's exciting research journey and highlighting our most recent work on the role of centrosomes in establishing proper sperm head-tail linkage.

67 **Centromere Polymorphisms in *Drosophila melanogaster*** Miraz A Sadi[1], Lucas Hemmer[2], Cécile Courret[1], Amanda M

Larracuente[1] [1]Department of Biology, University of Rochester, [2]University of Rochester

Centromeres are chromosomal regions where kinetochores assemble and spindle fibers attach to coordinate chromosome segregation during cell division. Despite their essential role, centromeres evolve rapidly across species. Although they are typically buried in highly repetitive genome regions, the role of DNA sequences in centromere function and specification is unclear, as they are epigenetically defined by the presence of the histone H3 variant CENP-A. To better understand the role of centromere-associated sequences in centromere function and evolution, we study centromere evolutionary dynamics within *Drosophila melanogaster* and examine the relationship between structural variation at the DNA level and functional variation in centromeric chromatin. We have recently shown that in *D. melanogaster* centromeres correspond to islands of retroelements flanked by simple satellite tandemly repeats. To ask if the centromere islands are conserved within *D. melanogaster*, we tested 5 strains with different geographical origins and compared them to the reference strain Iso1. We used long and short-read genomic DNA sequencing to determine the structural organization and CUT&Tag to identify CENP-A-enriched DNA in each strain. We identified the reference centromere islands, compared their organization and composition amongst the strains, and validated our observations with Fluorescence *In Situ* Hybridization (FISH) on mitotic chromosomes from larval brains using oligopaints specific to each centromere island. Our analyses reveal that both the dot and the X chromosome centromeres are polymorphic between strains and in each case, the reference centromere islands are absent from the genome. Thus here we discover structural DNA variation that is associated with a shift in centromeric chromatin, suggesting that the organization of the reference centromere islands is not essential for centromere function. However, centromeres may still have conserved features even when their organization is variable, as we find that CENP-A enriched sequences are similar across strains, including those with polymorphic centromeres. Altogether, our study highlights that the DNA sequences underlying centromeres are plastic and can be highly dynamic within species.

68 **Balanced inversions help maintain sexually antagonistic polymorphism** Christopher S McAllester, John E Pool Laboratory of Genetics, UW Madsion

Inversion polymorphisms are well documented across many taxa, despite the potential generation of unfit, unbalanced gametes from inversion heterozygotes. Inversions may fix because of linkage with beneficial alleles or due to drift, but many inversions are maintained at intermediate, in some cases clearly balanced frequencies, potentially by linking alleles that share conditional benefit. In African *Drosophila melanogaster*, paracentric inversions are common and many inversions are stably polymorphic throughout diverse African lowland habitats, suggesting the involvement of evolutionary forces beyond local adaptation. We hypothesize that balanced sexually antagonistic selection may be responsible for maintaining the stable polymorphism, in line with the active competition among *D. melanogaster* males and the potential for sexual antagonism. We used a novel forward population simulator with parameters based on *D. melanogaster* life history to model inversion evolution in a population under sexually antagonistic selection at infinite loci and with male reproductive skew. Simulations demonstrated (1) balanced polymorphism involving alleles with a range of antagonistic effects, (2) the persistence of such polymorphic alleles at many loci only under linkage due to competitive effects, and (3) the rise in frequency and stable persistence of inversions that establish such linkage associations between sets of sexually antagonistic alleles. In highly antagonistic simulations, some inversion arrangements are only in parents of one sex. We followed with an empirical exploration of the selection dynamics on inversions between a pooled Zambian paternal population and their embryo and aged adult offspring to detect correlations between the inversion status, viability and mating fitness. Results demonstrated non-neutral frequency changes, consistent with a complex fitness landscape in which only Inversion 3RK demonstrated a consistent tradeoff between male reproductive success and viability-longevity under the conditions and frequencies tested. This establishes the potential presence of this modeled dynamic in *D. melanogaster* inversions. This model has implications for sex chromosome evolution, as a segregating autosomal antagonistic inversion would likely have accumulated significant antagonistic character and benefit from linkage to a sex determining locus. Further, balancing selection upon epistatic haplotypes, particularly due to sexual or ecological antagonistic selection, may contribute significantly to genetic diversity and ongoing evolution and local adaptation in natural populations.

69 **The functional consequences of chromosomal inversions in local adaptation to high altitude in deer mice** Kelsie E Hunnicutt[1], Keely R Corder[2], Zachary A Cheviron[2], Jonathan P Velotta[3] [1]Department of Biological Sciences, University of Denver, [2]University of Montana, [3]University of Denver

Local adaptation is critical for species that occupy diverse and physiologically challenging habitats, but migration with non-adapted populations can challenge the establishment and maintenance of locally adapted alleles. Chromosomal inversions can facilitate adaptation by reducing gene flow of locally adapted regions of the genome. Deer mice (*Peromyscus maniculatus*) are a broadly distributed species with the largest altitudinal range of any North American mammal. High-altitude populations of deer mice face intense dual local selective pressures of extreme cold and low-oxygen availability which favors the evolution of system-wide physiological adaptations. However, local adaptation to high altitude in deer mice is challenged by ongoing

gene flow. We use two approaches to investigate how chromosomal inversions have contributed to local adaptation to high altitude in deer mice. First, we use a combination of whole genome and exome sequencing to characterize chromosomal inversions segregating in deer mice across an altitudinal cline ranging from ~400m (Lincoln, NE) to over 4000m (Mt. Evans, CO). Several inversions have been previously documented across North American deer mouse populations, but we find evidence of novel inversions unique to our altitudinal transect. We calculate cline centers and widths of segregating inversions and compare with clines of genes putatively under positive selection and neutral regions. We use four high-altitude populations (>3500m) to investigate parallel evolution of inversion genotypes and estimate population differentiation and the rate of molecular evolution in inverted and non-inverted regions. Second, we investigate the functional consequences of inversions by correlating gene expression variation with inversion genotype. We use RNASeq data from contrasts of highland and lowland deer mice facing hypoxic challenges across 9 tissues from 10 studies to investigate how inversions have shaped gene expression underlying diverse physiological processes including metabolism, thermogenesis, cardiovascular and pulmonary function, and reproduction. We establish which inversions repeatedly contribute to differential expression between highland and lowland deer mice across tissues and which genes are repeatedly involved across diverse physiological processes. Understanding the functional consequences of inversions will provide insight into the mechanisms of local adaptation and the ways in which structural genomic variation can hinder or promote these processes.

70 **Sex chromosome formation, expansion and turnover in the genus Rumex** Stephen Wright[1], Cassandre Pyne[2], Bianca Sacchi[2], Meng Yuan[2], Mark Hibbins[2], Jana Kružlicová[3], Markéta Bodláková[4], Václav Bačovský[4], Tyler Kent[5], Baharul Choudhury[2], Spencer Barrett[2] [1]Ecology and Evolutionary Biology, Univ Toronto, [2]University of Toronto, [3]Institute of Biophysics Czech Academy of Sciences, [4]Institute of Biophysics of the Czech Academy of Sciences, [5]university of Toronto

What evolutionary forces drive the expansion and turnover of sex chromosomes and what are the consequences? Here we investigate this question using a combination of comparative and population genomics in the genus Rumex, a plant system with highly heteromorphic and dynamic sex chromosomes. Genome assemblies of members of both dioecious and hermaphroditic species in the genus reveals very high rates of chromosomal rearrangements genome-wide, which has contributed to a complex and recurrent buildup of massive sex-linked regions in species with sex chromosomes, coupled with extensive gene silencing and loss over short timescales. Analysis of neo sex-linked regions suggest that transposable element invasion occurs early following sex- linkage, raising the possibility of their early contribution to gene silencing and inactivation. Population genomic analysis of the youngest sex-linked region in the genus provides evidence for a role for selective sweeps due to local adaptation in driving the spread of neo-sex chromosomes and the expansion of sex-linked regions.

71 **Enrichment of hard sweeps on the X chromosome across six *Drosophila* species** Mariana Harris[1], Bernard Kim[2], Nandita Garud[3] [1]Computational Medicine, University of California, Los Angeles, [2]Stanford University, [3]University of California, Los Angeles

The X chromosome is hemizygous in males, leaving it fully exposed one third of the time to the effects of natural selection and, thus, potentially subject to different evolutionary dynamics than autosomes. This is of particular interest given the potential importance of sex chromosomes in local adaptation, speciation, and sexual dimorphism. Here, we investigate the differences in the mode and tempo of adaptation on the X chromosome versus autosomes in multiple species of *Drosophila*. Specifically, we test the hypothesis that hard sweeps, expected when adaptation is gradual, are more common on the X chromosome due to there being a smaller effective population size and a reduction in standing genetic variation resulting from more efficient selection. By contrast, we predict that soft sweeps, expected when adaptation is rapid, are more common on the autosomes. In our recently published work, we find an enrichment of hard sweeps on the X chromosome relative to the autosomes in a North American population of *D. melanogaster*, confirming predictions we make from simulations. Now, we generalize these findings by analyzing diversity patterns across six *Drosophila* species, where we find regions with steep reductions in diversity and elevated haplotype homozygosity on the X chromosome as compared to autosomes. To assess if these signatures are consistent with positive selection, we simulate a wide variety of evolutionary scenarios, spanning variations in demography, mutation rate, recombination rate, background selection, soft sweeps, and hard sweeps, and find that the diversity patterns observed on the X are most consistent with a hard sweep model. Our results suggest that hard sweeps have played a significant role in shaping diversity patterns on the X chromosome across multiple species of *Drosophila*.

72 **Quantitative genetics of sex chromosome evolution** Pavitra Muralidhar University of Chicago

In organisms with separate sexes, the predominant mechanism of sex determination is the segregation of a designated pair of chromosomes at meiosis. While evolutionary transitions among sex chromosome systems have occurred frequently in certain taxa, in others, such as birds and mammals, the sex chromosome system displays long-term evolutionary stability. Many explanations have been proposed for this stasis, invoking, for example, accumulation on the sex-specific chromosome (Y/W) of recessive deleterious mutations or sexually antagonistic alleles. Here, I show that the ordinary operation of natural selection

on quantitative traits alone can strongly promote stability of the prevailing sex chromosome system.

Under stabilizing selection, the genome-wide genetic contribution to a quantitative trait – the 'additive genetic value' – remains close to the optimal trait value in each sex. However, the additive genetic values of individual chromosomes within the genome can drift over time, constrained only in that their sum must equal the optimal value. Therefore, over time, individual chromosomes can come to vary substantially in their average genetic values, in a delicate balancing act where chromosomes that happen to have drifted to high genetic values are complemented by chromosomes that have drifted to low genetic values.

A transition from one sex chromosome system to another necessarily involves the creation of novel sexual genotypes, potentially disrupting this genome-wide balancing act across chromosomes. I show that, under reasonable assumptions about the strength of selection and the genetic correlation between the sexes, novel sexual genotypes will deviate systematically from optimal phenotypic values and therefore suffer substantial fitness costs. These fitness costs impede—and with enough time prevent—the invasion of new sex-determining mechanisms.

The theory described above relies only on the presence of stabilizing selection on quantitative traits. As I show, the fitness costs to novel sexual genotypes—and therefore the barrier to transitions in the mechanism of sex determination—are exacerbated by (i) shifts in optimal phenotypic values, especially if these shifts are sexually antagonistic, and (ii) the simultaneous operation of stabilizing selection on multiple phenotypes. Finally, the 'chromosome-level' mechanism proposed here has implications for when Haldane's rule will most strongly manifest in hybrids between diverged species and populations.

73 **Patterns of genomic ancestry in the house mouse hybrid zone** Megan Frayer[1,2], Leslie Turner[3], Bettina Harr[4], Peicheng Jing[2], Bret Payseur[2] [1]Yale University, [2]University of Wisconsin-Madison, [3]University of Bath, [4]Max-Planck-Institut fuer Evolutionsbiologie

The history of hybridization is recorded in the ancestries of hybrid genomes. Natural selection and demography determine how the genomes of divergent lineages mix, shaping the genomic distributions of ancestries. Recombination in hybrids creates transition points between ancestries along chromosomes called junctions. Although junction patterns are known to be sensitive indicators of demography and selection, these patterns have yet to be characterized in a genome-wide manner in active hybrid zones. We used genome sequencing and probabilistic methods to reconstruct ancestry junctions across the genomes of house mice sampled from two populations from the European hybrid zone between *Mus musculus domesticus* and *M. m. musculus*. We report the identification of hundreds of thousands of junctions (an individual average of 10 junctions per Megabase) and describe junction sharing across individuals and populations using a novel metric, the junction frequency spectrum. Despite being separated by only 7.4 km in the center of the hybrid zone, the two populations display stark differences in genome-wide junction distributions, suggesting a crucial role for demographic processes. We pinpoint regions of the genome that show unusual junction patterns indicative of selection against hybrid incompatibility loci that could underlie reproductive barriers between *M. m. domesticus* and *M. m. musculus*. Our results suggest that the genome-wide distribution of junctions is a promising target for inference of selection and demography in hybrid zones.

74 **Evolutionary origin of the recent allotetraploid *Mimulus sookensis*** Makenzie Whitener, Andrea Sweigart University of Georgia

Polyploidy occurs across the tree of life and is especially common in plants. Because newly formed cytotypes are often incompatible with their progenitors, polyploidy is also said to trigger "instantaneous" speciation. If a polyploid can self-fertilize or reproduce asexually, it is even possible for one individual to produce an entirely new lineage. How often this extreme scenario occurs is unclear, with most studies of wild polyploids reporting multiple origins. Here, we explore the evolutionary origin of the wild allotetraploid *Mimulus sookensis,* which was formed through hybridization between self-compatible, diploid species in the *Mimulus guttatus* complex. We generate a chromosome-scale reference assembly for *M. sookensis* and define its distinct subgenomes. Despite previous reports suggesting multiple origins of this highly selfing polyploid, we discover patterns of population genomic variation that provide unambiguous support for a single origin, which we estimate occurred ~71,000 years ago. One *M. sookensis* subgenome is clearly derived from the selfer *M. nasutus*, which, based on organellar variation, also appears to be the maternal progenitor. The ancestor of the other subgenome is less certain, but it shares variation with both *M. decorus* and *M. guttatus*, two outcrossing diploids that overlap broadly with *M. sookensis*. This study establishes *M. sookensis* as an example of instantaneous speciation, likely facilitated by the polyploid's predisposition to self-fertilize. We also perform RNAseq on newly synthesized and wild *M. sookensis* to investigate the initial and longer-term regulatory effects of whole genome duplication.

75 **Drying Without Dying: Small Metabolites Counteract Proteotoxicity During Desiccation** Alejandra Arroyo, Sheila Ferer, Gabbriella Amador, Jailene Martinez, Alina Vane, Hugo Tapia CSUCI

Most biological processes require water to function, so desiccation can be distressing for many living organisms. Desiccation tolerant organisms, commonly termed anhydrobiotes, can lose significant amounts of water and still function normally once rehydrated. The exact stress(es) that cause lethality in desiccation sensitive organisms and how the lethal stresses are mitigated in desiccation tolerant organisms are poorly understood. We establish that trehalose cooperates with stress-induced glycerol and glycogen in the establishment of desiccation tolerance, its prolonged maintenance, and susceptibility to secondary stresses of heat and cold. We provide evidence that desiccation induces protein misfolding of a luciferase reporter and protein aggregation as evidenced by its ability to cure prions through the hyper-aggregation of prion seeds. Trehalose mitigates excessive protein aggregation but not protein misfolding during desiccation. We suggest that protein aggregation is a persistent stress during desiccation that is mitigated by trehalose.

76 **Impact of α-arrestins on organelle function and cellular metabolism** Elif Filiztekin[1], Annette Chiang[1], Nejla Ozbaki-Yagan[1], Stacy Gelhaus Wendell[1], Florian Fröhlich[2], Allyson F O'Donnell[1] [1]Department of Biological Sciences, University of Pittsburgh, [2]Department of Biology, Osnabrück University

To survive changes in nutrient availability or stressors, cells rearrange their membrane proteome by stimulating vesicle-mediated protein trafficking. The α-arrestins are master regulators of protein trafficking, helping to selectively relocalize membrane proteins. The best-studied class of membrane protein regulated by the α-arrestins are the amino acid transporters (AATs), which allow uptake of amino acids from the extracellular environment. The loss of α-arrestins causes aberrant retention of amino acid transporters at the plasma membrane, which may lead to excess amino acid accumulation in the cell.

Using metabolomics approaches, we find that intracellular concentrations of select amino acids are increased in cells lacking α-arrestins. For instance, we find that cells lacking α-arrestin Art1, a known regulator of the arginine and lysine transporters Can1 and Lyp1, respectively, have elevated intracellular arginine and lysine. What are the consequences of excess amino acids on cellular physiology? Cells lacking Art1 have fragmented mitochondria with reduced membrane potential and defective cellular respiration, as evidenced by their inability to grow on glycerol. We show that these defects can be restored by negating the cells' ability to take up arginine and lysine, either by growing them in the absence of exogenous arginine and lysine or by deletion of the Can1 and Lyp1 transporters. This defective mitochondrial function is linked to impaired vacuole function and morphology. The vacuole proteome and lipid composition are dysregulated in cells lacking α-arrestin Art1, and this contributes to defective mitochondrial function and amino acid imbalances. Our research demonstrates an exciting new link between the maintenance of amino acid homeostasis and regulation of polyphosphate metabolism. Over 80% of cellular arginine is stored in vacuoles, and to increase arginine storage in this organelle, cells lacking Art1 upregulate polyphosphate synthesis. Early studies of polyphosphate demonstrated that it serves as a counter ion in the vacuole, enabling increased storage of the cationic arginine. These dramatic shifts in cellular physiology when α-arrestin Art1 is lost help define the interconnection between cellular metabolism and organelle function.

77 **Glyphosate as an amino acid mimic and the role of human glutamate transporters in glyphosate import** Dionysios Patriarcheas, Jennifer E Gallagher West Virginia University

Glyphosate is the globally predominant herbicide found in RoundUp® and understanding its cellular mechanisms of action is crucial. As an inhibitor of the **shikimate** pathway, glyphosate blocks the biosynthesis of aromatic amino acids and is thus perceived as harmless to humans, who rely on their diet for their intake. Surprisingly, studies have reported various **toxic effects** in animals caused by exposure to high concentrations of glyphosate, including neurotoxicity, reproductive toxicity, and gut microbiome disruption. However, the **mechanism** through which glyphosate is **transported** has yet to be definitively demonstrated. In *S. cerevisiae* glyphosate acts as an amino acid **mimic** and can utilize **Dip5**, a glutamate/aspartate permease, to enter the cells. Knocking out *dip5* confers resistance to glyphosate. Our findings reveal that knocking out *agc1/ymc1*, two **mitochondrial** glutamate **transporters**, appears to significantly increase resistance to glyphosate, which could hint at mitochondrial off-target effects. Previous work from our group has highlighted the central role of mitochondrial metabolism in response to glyphosate. To identify the human transporter responsible for glyphosate transport, we further heterologously express **human glutamate transporters** in *DIP5* and *AGC1/YMC1* knockout yeast and assess their resistance to glyphosate. Human transporters that can transport glyphosate will cause mutant yeast expressing this transporter to be glyphosate-sensitive. Understanding which transporter is responsible for glyphosate import will guide our understanding in identifying the type of tissue that is affected the most through the expression patterns of the transporter. In order to verify that Dip5 and other glutamate transporters are responsible for the cellular import of glyphosate **metabolite** extracts of wild-type and *dip5Δ* will be analyzed through **mass spectrometry**. We predict that the glyphosate-resistant *dip5* mutants will have lower intracellular levels of glyphosate compared to wildtype cells supplemented with aromatic amino acids. Wild-type cells supplemented with aromatic amino acids are able to grow because they bypass the shikimate pathway despite having intracellular glyphosate. Our aim is to lay the foundation for understanding **glyphosate toxicity** in humans by elucidating glutamate transporter roles in resistance and toxicity.

78 **Single-cell RNA sequencing data reveal new energetic constraints on making a (microbial) cell** Leandra Brettner[1], Rachel Eder[2], Kerry Geiler-Samerotte[2] [1]Biodesign Center for Mechanisms of Evolution, Arizona State University, [2]Arizona State University

Previous work has established that the number of ribosomes in a cell represents the primary constraint on how fast cells can grow and divide. This constraint, called a "growth law" is apparent across the tree of life. It is obvious why the number of ribosomes sets a lower limit on how fast cells can make more cells: ribosomes are required to make all the cellular components. But there may exist other constraints that prevent cells from reaching their growth potential even when enough ribosomes are present. These additional constraints may have been missed by previous work because that work largely focuses on cells growing in steady nutrient levels in chemostats. These experiments may miss growth-limiting parameters that dictate the limits of cellular evolution because natural populations of cells are prone to experience feast and famine scenarios. Here, we explore the extent to which ribosome content predicts growth rate in conditions where microbial cells are slowly consuming all available nutrients. It is well known that microbial cell populations "bet hedge" to contend with famine, whereby different cells deploy different strategies in response to nutrient depletion. Therefore, we use a single cell approach to measure ribosomal content. Though we confirm that the growth law holds true for populations of cells that are depleting their nutrients, we find that ribosomal content alone is a poor predictor of the growth rate of single cells. We observe enormous variance in the ribosomal content from cell to cell, and opposite expectations, this variance decreases with decreasing nutrient availability. By analyzing the full transcriptional profiles of cells with different ribosomal content and different growth rates, we begin to reveal additional constraints on making a cell, for example, the degree to which resources are divested towards stress responses. This study, and the novel biology we learned, were made possible because we utilized a new single-cell RNA seq approach that allows sampling the transcriptomes of thousands of microbial cells. We applied this approach to cells of two different microbes, the eukaryotic yeast, S. cerevisiae, and the prokaryote, B. subtilis, both of which appear to grow at rates dictated by constraints in addition to ribosome number.

79 **Divergence of TORC1-mediated stress response leads to novel acquired stress resistance in a pathogenic yeast** Jinye Liang, Hanxi Tang, Lindsey Snyder, Bin He, christopher youngstrom Biology Department, the University of Iowa

Acquired stress resistance (ASR) enables organisms to prepare for environmental changes that occur after an initial stressor. However, the genetic basis for ASR and how the underlying network evolved remain poorly understood. In this study, we discovered that a short phosphate starvation induces oxidative stress response (OSR) genes in the pathogenic yeast *C. glabrata* and protects it against a severe H_2O_2 stress; the same treatment, however, provides little benefit in the low pathogenic-potential relative, *S. cerevisiae*. This ASR involves the same transcription factors (TFs) as the OSR, but with different combinatorial logics. We show that Target-of-Rapamycin Complex 1 (TORC1) is differentially inhibited by phosphate starvation in the two species and contributes to the ASR via its proximal effector, Sch9. Therefore, evolution of the phosphate starvation-induced ASR involves the rewiring of TORC1's response to phosphate limitation and the repurposing of TF-target gene networks for the OSR using new regulatory logics.

80 **Elucidating a Novel Role for Septins During High Temperature Stress Response in *Cryptococcus neoformans*** Stephani Martinez[1], Lukasz Kozubowski[2] [1]Clemson University, [2]Genetics and Biochemistry, Clemson University

The pathogenic yeast *Cryptococcus neoformans* adapts to changes in temperature upon entering human host. *C. neoformans* strains lacking septin proteins Cdc3 or Cdc12 are viable at 25°C but fail to proliferate at 37°C and are likely avirulent. Septins are conserved filament-forming GTP-ases that assemble as higher order complexes at the cell cortex to support cytokinesis and morphogenesis in fungal and animal cells. In *C. neoformans*, four septin homologues, Cdc3, Cdc10, Cdc11 and Cdc12 assemble at the mother-bud neck and contribute to cytokinesis via poorly characterized mechanism. We observed that septin complex also assembles at the plasma membrane specifically when cells are exposed to 37°C and hypothesize that septins play stress-related functions in *C. neoformans*. The *C. neoformans* septin interactome remains unknown. In this study, tandem-mass spectrometry was utilized to identify proteins that associate with septins Cdc3 and Cdc10 in *C. neoformans at either 25 or 37°C. Cdc3 was used as a bait since it's an integral part of the septin complex. Cdc10 is presumably dispensable for septin complex formation and served as "bait" to identify proteins that interact specifically with Cdc10. 196 and 40 proteins, including the remaining 3 septins, were identified based on Cdc3 pull down, at 25 and* 37°C, respectively. In contrast, the total number of Cdc10 binding partners was 112 and 46 at 25 & 37°C, respectively. The Cdc10 interactome also included the entire septin complex at both 25 & 37°C. Furthermore, 14 proteins including the entire septin complex were found as interacting with both Cdc3 and Cdc10 at both 25 & 37°C. Therefore, those 14 proteins might represent the core proteins of the septin complex interactome that is independent of thermal stress for binding. In addition, 9 proteins were identified as binding partners of both Cdc3 & Cdc10 exclusively at 37°C. Those proteins specific to heat stress are involved in GDP-GTP exchange, mRNA processing, microtubule binding, and plasma membrane transport. The association of both septins with a plasma membrane transporter (CNAG_00730) provides an insight into the role of septins during heat

stress. CNAG_00730 is the homologue of 8 ABC transporters in *S. cerevisiae*'s, which are involved in drug resistance, cellular detoxification, general stress response, and sterol uptake when sterol biosynthesis is compromised. Thus, those 9 candidate proteins may represent septin interactome that reflects adaptation of *C. neoformans* to host temperature.

81 **A few essential genetic loci distinguish Penstemon species with flowers adapted to pollination by bees or hummingbirds** Carolyn Wessinger[1], John Kelly[2], Lena Hileman[2] [1]Biological Sciences, University of South Carolina, [2]EEB, University of Kansas

In the formation of species, adaptation by natural selection generates distinct combinations of traits that function well together. The maintenance of adaptive trait combinations in the face of gene flow depends on the strength and nature of selection acting on the underlying genetic loci. Floral pollination syndromes exemplify the evolution of trait combinations adaptive for particular pollinators. The North American wildflower genus Penstemon displays remarkable floral syndrome convergence, with at least 20 separate lineages that have evolved from ancestral bee pollination syndrome (wide blue-purple flowers that present a landing platform for bees and small amounts of nectar) to hummingbird pollination syndrome (bright red narrowly tubular flowers offering copious nectar). Related taxa that differ in floral syndrome offer an attractive opportunity to examine the genomic basis of complex trait divergence. In this study, we characterized genomic divergence among populations of a Penstemon species complex that includes both bee and hummingbird floral syndromes. Field plants are easily classified into species based on phenotypic differences and hybrids displaying intermediate floral syndromes are rare. Despite unambiguous phenotypic differences, genome-wide differentiation between species is minimal. Hummingbird-adapted populations are more genetically similar to nearby bee-adapted populations than to geographically distant hummingbird-adapted populations, in terms of genome-wide dXY. However, a small number of genetic loci are strongly differentiated between species. These "species-diagnostic loci," which appear to have nearly fixed differences between pollination syndromes, are sprinkled throughout the genome in high recombination regions. Several map closely to previously established floral trait quantitative trait loci (QTLs). The striking difference between the diagnostic loci and the genome as whole suggests strong selection to maintain distinct combinations of traits, but with sufficient gene flow to homogenize the genomic background. A surprisingly small number of alleles confer phenotypic differences that form the basis of species identity in this species complex.

82 **Integrating comparative and quantitative genetics to accelerate breeding efforts** John Lovell Genome Sequencing Center, HudsonAlpha Institute for Biotechnology

A single haploid reference genome provides breeders with the resources to connect alleles to traits — a significant step towards accelerating crop improvement through biotechnology. However, breeding programs often leverage highly diverged germplasm, which contain large-scale variants that are not readily identified by a single reference genome but may underlie heritable variation in key traits. To assist breeding and gene discovery efforts, we have built multiple reference genomes in many species across the diversity of plants. In concert with these resources, we developed a comparative genomics pipeline "GENESPACE" to connect and explore putatively functional variants that may affect agricultural productivity. Here, we present some of these variants and describe how comparative and quantitative genomics can be integrated to accelerate breeding efforts in maize, switchgrass, pecan, and sorghum.

83 **The post-domestication history of rice: insights from herbarium genomes and ancestral recombination graphs** Ornob Alam[1], Michael Purugganan[2] [1]Biology, New York University (on leave from Independent University, Bangladesh), [2]Biology, New York University

The dispersal of rice (Oryza sativa) following domestication influenced massive social and cultural changes across South, East, and Southeast (SE) Asia. While recent resequencing of traditional rice landraces from across Asia have revealed possible routes of dispersal across Southeast Asia, the early history and spread of rice across and out of China remain largely unresolved. This is mainly because of sparse sampling of traditional landraces of japonica rice — the ancestral rice domesticate — from China as these have largely been replaced by modern improved or hybrid varieties and indica rice. We addressed this dearth of sampling by collecting historical specimens of domesticated rice from herbaria in France, the UK, and the US, originally sampled from China and other parts of Asia by botanists in the 19th and early 20th century. Here, we performed whole-genome resequencing of 96 herbarium rice specimens and analyzed them alongside a previously published set of 367 japonica rice landraces. We used ancestral recombination graph-based approaches to reconstruct the demography and routes of dispersal of japonica rice following domestication in China. We also utilized the ancestral recombination graphs to investigate the evolutionary history of adaptive and functional variants — including those involved in flowering time, immunity, and taste— in populations of japonica rice, revealing widespread local adaptation as rice spread to different regions, climates, and latitudes.

84 **The Loaded Potato Genome** Sapphire Coronejo[1], Xiaoxi Meng[2], Paul Bethke[3], David Douches[4], Joshua Parsons[5], Vidyasagar Sathuvalli[6], Ek Han Tan[7], Jeffrey B Endelman[8], C. Robin Buell[9], Laura M Shannon[1] [1]University of Minnesota, [2]St.

Jude's Children's Research Hospital, [3]USDA-ARS, [4]Michigan State University, [5]Pepsi Co, [6]Oregon State University, [7]University of Maine, [8]University of Wisconsin, [9]University of Georgia

Potato is essential to cultures and cuisines around the world. However, potato is less well understood than other staples in part due to its highly heterozygous autotetraploid genome. The first eight phased autotetraploid potato genomes were sequenced in 2022. Although these genomes were informative they mostly allowed us to draw conclusions about individual potato varieties, rather than US cultivated potato as a population. Prickle pollination of 4X mothers with a diploid inducer allows us to generate diploid potato plants with two chromosomes from the 4X parent. These individuals give us the opportunity to sequence tetraploid haplotypes using technology for diploids. Through prickle pollination we have generated a panel of 100 diploid potatoes arising from 60 tetraploid mothers and sequenced them to ~20X using Illumina short reads. Although there is some structure arising from differences in market classes, in general population structure is minimal. While observed heterozygosity is slightly higher than nucleotide diversity suggesting some balancing selection in particular on chromosomes 6 and 10, overall population level diversity is high. Among these 100 phased genomes 12 coding sequences are fixed while 34 have 200 alleles. The average number of alleles per loci is 27.86. This suggests, the primary driver of heterozygosity in potato is population level diversity rather than balancing selection. Multiple factors contribute to potato diversity including the relaxation of selection on individual alleles in tetraploids leading to a surplus of deleterious alleles, extensive introgression from 20+ wild species, and transposons. Understanding these various contributors to the potato genome provides insight into cultivated potato evolution and directions for potato improvement as the crop is reinvented as a diploid.

85 **The Barley Cytonuclear Multi-Parent Population (CMPP) as a Novel Resource for Dissecting Cytonuclear Interactions** Schewach Bodenheimer[1,2], Eyal Bdolach[3], Lalit Tiwari[1], Tapish Pawar[4], Joseph Tilstra[5], Gazala Ameen[4], Eyal Fridman[1] [1]The Institute of Plant Sciences, Volcani Agricultural Research Organization (ARO), [2]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, [3]THe Institute of Plant Sciences, Volcani Agricultural Research Organization (ARO), [4]Department of Agronomy, Horticulture, & Plant Science, South Dakota State University, [5]South Dakota State University

Wild crop relatives possess a wealth of genetic diversity essential for crop improvement, but harnessing this diversity is hampered by the complex genetics of key traits. Cytonuclear interactions (CNIs) between cytoplasmic and nuclear genomic loci may play a critical role in this complexity, but the study of their additive and interactive effects is limited by current population infrastructures and computational tools. We address this challenge by developing the first plant cytonuclear multi-parent population (CMPP) in barley to serve as a unique resource for dissecting CNIs. The CMPP comprises 968 homozygous doubled haploid lines derived from F1 directional hybrids between 10 wild (*Hordeum vulgare ssp. spontaneum*) accessions and a common cultivar (cv. Noga). The ten wild donors represent adaptation to the diverse ecogeography of the southern Levant, a hotspot of allelic diversity. The design of the CMPP, which is a slight modification of the Nested Association Mapping (NAM) concept, includes a reciprocal second backcross and thus involves segregation of both wild cytoplasmic and nuclear genomes in each of the bi-parental subpopulations. We refined a protocol for chloroplast DNA isolation for analysis by next-generation sequencing and found non-synonymous mutations in chloroplast genes. The nuclear genome was characterized through skim coverage sequencing, with imputation from high-coverage founder lines, and with 50K SNP genotyping for easy alignment with previous and ongoing research in this model crop plant. Our population genetics analysis shows, as expected, an average of 12.6% nuclear introgression from the wild donors among the CMPP lines. Analysis of the field phenotype shows significant cytoplasmic effects on grain dimensionality and flowering time in some of the CMPP families. Efforts are now focused on identifying the genetic loci interacting with different cytoplasmic backgrounds and conducting multi-environment field trials to assess the stability of CNI-QTL underlying yield related traits. Ultimately, the CMPP offers a novel genetic resource for enhancing our understanding of nuclear-cytoplasmic genome interactions, and it will be available with its genomic and phenotypic information in the b1kCMPP public database.

86 **Elucidating the genetic architecture governing cytonuclear genomic stoichiometry** Evita Chee[1], Joel Sharbrough[2] [1]Biology/Biotechnology, New Mexico Institute of Mining & Technology, [2]Biology, New Institute of Mining & Technology

Plant genomes are partitioned into three separate cellular compartments: the nucleus, mitochondria, and chloroplasts. Nuclear genomes are bi-parentally inherited via sexual reproduction while the two cytoplasmic genomes are inherited uniparentally. Together, these genomes encode proteins that must interact with one another, in highly specific ratios, to assemble multi-subunit enzyme complexes that carry out essential plant processes like oxidative phosphorylation (OXPHOS) and photosynthesis. The genes encoding nuclear and organelle gene products that interact (*i.e.,* cytonuclear interactions) are known to be highly dosage sensitive, as cytoplasmic gene copy number is upregulated in polyploids, and nuclear-encoded genes whose products are targeted to the chloroplast or mitochondria are among the first to re-diploidize following whole-genome duplication events. The genetic architecture underlying the phenotypic consequences that proceed from

the delicately balanced cytonuclear stoichiometry of plants are entirely unknown. We therefore designed crosses between accessions of *A. thaliana* that exhibit either high or low cytoplasmic genome copy numbers (*i.e*., high x low; low x high) to identify candidate gene regions underlying variation in cytonuclear stoichiometry. We confirmed the success of these crosses using RFLP markers on DNA extracted from F_1s and selfed the F_1s to produce recombinant F_2 offspring. We reared F_2 progeny in a common garden and assayed photosynthetic activity, quantified ratios of cytoplasmic:nuclear RNA and DNA, and plan to sequence whole genomes on an Illumina NovaSeq to perform QTL analysis. As part of this analysis, we also modeled the relationships between cytonuclear ratios of DNA and RNA with photosynthetic traits to determine the effects of variation in cytonuclear stoichiometry on photosynthetic performance. In the future, we will target candidate genes with CRISPR/Cas gene editing to knock genes out (or down) to evaluate functional contributions to cytonuclear stoichiometry. In sum, this project will provide valuable insights into the mechanistic regulation responsible for changes in cytoplasmic genome copy number. More broadly, understanding the effects of cytoplasmic genome copy number variation on photosynthesis represents a key and unsolved mystery in plant biology.

87 **The genetic basis of complex traits in an over-wintering *Arabidopsis thaliana* common garden** Amanda L Peake, John R Stinchcombe Ecology and Evolutionary Biology, University of Toronto

Characterizing the genetic basis of ecologically important traits is one of the main goals of evolutionary biology. Common garden experiments conducted in realistic conditions can be extremely beneficial for identifying ecologically relevant genetic variants given that complex quantitative traits are influenced by an individual's genetic make up as well as their environment. *Arabidopsis thaliana's* abundance of genomic resources and impressive collection of accessions sampled from across the species geographic range make the species ideal for studying the relationships between genetic variation, phenotypic diversity, and fitness. We grew 500 *A. thaliana* accessions, collected from North Africa, Europe, and Asia by the 1001 Genomes Consortium (2016), in an over-wintering common garden experiment in Toronto from September 2022 to July 2023. We measured a variety of ecologically important traits including early life growth rate, flowering time, flowering duration, number of leaves, rosette diameter, winter survival, plant height, branching architecture, above-ground dry biomass, and fruit number. Our goals are 1) to identify novel ecologically relevant genetic variants, 2) determine the effect of Gene-by-Environment interactions (GxE) on the phenotypic traits in *A. thaliana*, and 3) determine the strength of selection acting on the traits during the 2022-2023 growing season. To determine how GxE may be influencing phenotypic variation, we compared our data with other studies that have previously grown the same accessions in growth chambers kept at a constant temperature of 10°C and 16°C. According to our preliminary results, we find an effect of genotype, environment, and GxE when we compare flowering time in our overwintering common garden experiment to flowering time in both growth chambers and we find no difference in flowering time between the two growth chambers. We conducted a Genome Wide Association Study (GWAS) to determine the genetic basis of flowering time in all three conditions. We found a total of 5 genomic regions associated with flowering time in our outdoor common garden experiment one of which overlaps with the well-known flowering time gene *FRIGIDA*. We then compared the genomic regions associated with flowering time in our outdoor common garden experiment to the genomic regions associated with flowering time in the growth chambers. Unsurprisingly, we found overlapping GWAS hits between the two growth chambers. We did however find different genomic regions associated with flowering time in our overwintering common garden experiment compared to the 10°C and 16°C growth chambers. Therefore, phenotyping *A. thaliana* in a more ecologically realistic environment led to novel GWAS hits. We are currently conducting similar analyses for the other traits we measured and we will also be using fruit number as a proxy for fitness to calculate the strength of selection.

88 **Unravelling the molecular genetics of root system architecture in wheat (*Triticum aestivum* L.)** Tanushree Halder[1,2], Guijun Yan[2], Yinglong Chen[2], Hui Liu[2], Kadambot H. M. Siddique[2] [1]Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, [2]The University of Western Australia

Background: Wheat (*Triticum aestivum* L.) production in the world is challenged by different biotic and abiotic stresses, which are increasing with climate change. An improved root system is essential for adaptability and sustainable wheat production. Unravelling the molecular genetics—quantitative trait loci (QTL), candidate genes and proteins—of root traits is essential wheat root trait improvement through breeding.

Materials and methods: 103 recombinant inbred lines (RILs) RILs of Synthetic W7984 × Opata M85 and 14 pairs of near-isogenic lines (NILs: pairs of genetically identical lines except for genomic regions (GRs)) of wheat were used to phenotype root traits in a semi-hydroponic system. Composite interval mapping method was used to discover QTL for root traits from RILs. Candidate genes and proteins for root traits were identified from NILs through and genotype-phenotype association analysis, and label-free proteomics, respectively.

Results: The RILs exhibited significant variation in root traits; 14 QTL for eight root traits were identified mainly on chromosome

groups 5, 6 and 7. Important QTL for shallow root (*Q.rd.uwa.7BL*: *Xbarc50*) and high RM (*Q.rm.uwa.6AS*: *Xgwm334*) were validated in two independent F2 populations of Synthetic W7984 × Chara and Opata M85 × Cascade, respectively. Separately, 10 of 14 NIL pairs showed significant variation between their isolines for root traits; 15 putative candidate genes for root traits, including outstanding genes *TraesCS4A02G185300* and *TraesCS4A02G442700* encoding UDP-glycosyltransferase and *TraesCS4A02G330900* encoding leucine-rich repeat receptor-like protein kinase were identified on targeting GRs on chromosomes 4BS, 4BL, 4AS, and 7AL of NILs. Furthermore, three candidate protein biomarkers for total root length and root dry mass in NIL pairs targeting GRs on chromosomes 4A and 7A—asparagine synthetase (*TraesCS4A02G109900*), signal recognition particle 19 kDa protein (*TraesCS7A02G333600*) and 3,4-dihydroxy-2-butanone 4-phosphate synthase (*TraesCS7A02G415600*)—with consistent gene expressions at protein and mRNA transcription (qRT-PCR) levels were identified.

Conclusion: Our research provides an improved understanding of molecular control of root traits and potential for marker-assisted root breeding in wheat.

89 **A noncanonical GTPase signaling mechanism controls exit from mitosis in *Saccharomyces cerevisiae*** Xiaoxue Zhou[1,2], Stephen P Bell[1], Angelika Amon[1] [1]Massachusetts Institute of Technology, [2]New York University

In the budding yeast *S. cerevisiae*, exit from mitosis is coupled to nuclear/spindle position to ensure successful genome partitioning between mother and daughter cell (bud). This coupling occurs through a GTPase signaling cascade known as the mitotic exit network (MEN). Only when the anaphase spindle is positioned correctly along the mother-bud axis is the MEN activated to promote exit from mitosis. The MEN senses spindle position via a Ras-like small GTPase Tem1. How the GTP/GDP cycle of Tem1 couples the status of spindle position to the activation of its effector protein Cdc15 is not fully understood. Tem1 primarily localizes to the spindle pole body (SPB, yeast equivalent of centrosome) that migrates into the bud when the anaphase spindle is correctly positioned. In contrast, when the spindle is mispositioned the SPB localization of Tem1 is minimized. Here, we show that only the GTP bound Tem1 (Tem1-GTP) localizes to the SPB and, contrary to prior assumptions, Tem1's nucleotide state does not change upon MEN activation. More importantly, by artificially tethering Tem1 to the SPB, we demonstrate that the essential function of Tem1-GTP is to localize Tem1 to the SPB. Localization to the SPB mainly serves to create a high local concentration of Tem1 to activate Cdc15, as we could bypass this essential localization by concentrating Tem1 in the cytoplasm with genetically encoded multimeric nanoparticles (GEMs). We propose that rather than modulating Tem1's nucleotide state, spindle position regulates Tem1-Cdc15 interaction (and thus Cdc15 activation) by controlling Tem1's effective concentration through regulating its localization. Overall, our study reveals a distinct localization/concentration-based GTPase signaling mechanism for Tem1 that differs considerably from the canonical Ras-like GTPase signaling paradigm, in which the GTPase functions as a molecular switch with its nucleotide state modulated to regulate effector binding and activation.

90 **The Evolution of an Inversion Supergene in Deer Mice** Olivia Harringmeyer, Shuonan He, Hopi Hoekstra Harvard University

Supergenes, sets of genetic loci that are inherited together, can facilitate local adaptation through linking multiple adaptive traits. Supergenes are often associated with chromosomal inversions, since inversions link genetic loci through suppressing recombination when heterozygous. Despite growing evidence that inversion supergenes play an important role in adaptation, little is known about how inversions become supergenes. Here, we address two major open questions about the evolution of inversion supergenes. First, how do inversions influence adaptive traits? Second, do inversions become supergenes over time, or immediately upon their formation? To address these questions, we focus on a 40-Mb inversion supergene in the deer mouse (*Peromyscus maniculatus*) that is strongly associated with long tails and dark coats, two traits important for local adaptation to forest habitats. To dissect the molecular basis of the inversion supergene, we first isolated the inversion in a congenic mouse line, enabling quantification of the phenotypic effects of the inversion, on its own, across sibling mice. For coat color, we found that the inversion darkens mouse coats by reducing the expression of *Agouti*, the gene responsible for inducing the switch between dark and light pigment production. The inversion is located on a different chromosome from *Agouti*, suggesting that the inversion affects coat color through causing *trans* regulatory effects. For tails, we found that the inversion lengthens mouse tails by increasing the number of chondrocytes in tail vertebral growth plates. Using a forward genetic cross, we then fine-mapped the tail locus to a narrow 1-Mb region in the center of the inversion, in which we identified *Npr3* as the top candidate gene driving the tail length effects. These results suggest that mutation(s) harbored within the inversion, rather than the inversion breakpoints themselves, cause the inversion's effects on tail length. Finally, we investigated the inversion's phenotypic effects across the species range and found that the inversion consistently affects coat color, but only increases tail length in a portion of its range, indicating that the inversion acts as a supergene in some, but not all, populations in which it occurs. Moreover, we found that the inversion likely became a supergene over time, through the accumulation of the tail length mutation(s). Together, these results reveal how an inversion can alter two different developmental pathways associated with two distinct adaptive traits, becoming a supergene over time through the accumulation of adaptive loci.

91 **Mycobacterial effector EsxM alters macrophage dynamics via the host actin cytoskeleton** Mollie I Sweeney[1], Joseph W Saelens[2], Gopinath Viswanathan[2], Ana María Xet-Mull[2], Carson E Carranza[2], Jörn Coers[2], Jason E Stout[2], Clare M Smith[2], David M Tobin[2] [1]Molecular genetics and microbiology, Duke University, [2]Duke University

Mycobacterium tuberculosis (*Mtb*), the human-adapted pathogen that causes tuberculosis disease, has subverted the immune system to become one of the deadliest pathogens in history. We use the zebrafish-*Mycobacterium marinum* model of TB to study mycobacterial pathogenesis. We identified a secreted *Mtb* effector, EsxM, that induces dramatic phenotypes in infected cells. Clinically, *Mtb* strains that express full-length EsxM cause a high rate of disseminated disease. *M. marinum* infection experiments in both larval and juvenile zebrafish demonstrate that full-length EsxM-expressing *M. marinum* possesses an increased capacity for dissemination from established sites of infection and a tropism for bone infection, consistent with human clinical data. Using heterologous expression of either the modern, truncated version of EsxM or the ancient, full-length EsxM in zebrafish macrophages, we have shown that the full-length effector induces increased motility and migration of macrophages to a wound site. In human cells, we find that infection with EsxM-expressing *Mtb* leads to dramatic filopodial projections, F-actin and Arp2/3 rearrangement, and increased cell migration when compared to infection with *Mtb* strains lacking the effector. We identified a putative interacting partner for EsxM, a subunit of the host WASH complex, and developed a zebrafish *WASHC4* knockout line to test whether the increased macrophage migration and subcellular changes induced by EsxM are mediated via the WASH complex. *M. marinum*-infected mutant fish experience higher infection burdens and increased dissemination. Macrophages in mutant fish migrate at a higher rate, revealing a role for the WASH complex in cell motility. In a mouse *Mtb* infection model, disruption of the WASH complex leads to increased lung and spleen burden, demonstrating the importance of the complex in the context of mammalian mycobacterial infection. Bone marrow derived macrophages from mutant mice display unique actin localization patterns, including strong cortical actin bands, perinuclear localization of actin, and a general lack of cell polarization. Overall, we have identified a secreted mycobacterial effector that promotes dissemination of disease via alterations in the cytoskeleton of infected host macrophages. Our investigations into the putative host target, WASHC4, have implicated the WASH complex in macrophage migration and restriction of mycobacterial disease.

92 **Host genetics, microbiome composition and addiction/addiction-related behavior in mice.** Jason A Bubier[1], Dong-Bing Tran[2], Asaf Peer[2], Hoan Nguyen[2], Robyn Ball[2], Belinda Cornes[2], Jane Adams[3], Hao He[2], Vivek Philip[1], George Weinstock[2], Elissa J Chesler[1], Yanjiao Zhou[4] [1]Center for Addiction Biology, The Jackson Laboratory, [2]The Jackson Laboratory, [3]Northeastern, [4]Medicine, UConn Health

Cocaine use disorder (CUD) is a chronic disease characterized by compulsive drug seeking and use, leading to substantial adverse outcomes. CUD is highly heritable (>70%), and many genes have been associated with addiction. However, these only account for a small proportion of the observed variation, suggesting a role for many other biological and environmental factors. Accumulating evidence supports that the gut microbiome through the gut-brain axis plays a significant role in the behavioral responses to cocaine. Using the advanced mouse populations of the Diversity Outbred (DO)and Collaborative Cross (CC), our objective was to understand the interaction of the microbiome and host genetics in addiction or addiction-related behaviors. We hypothesize that host genetics influences behavior by altering the composition of the gut microbiome. When studied under a controlled diet, the microbiome's composition in animal models is a heritable genetic trait. Leveraging the data collected by the P50 Center for Systems Neurogenetics of Addiction, we used fecal samples and phenotype data from DO mice to test our hypothesis. We have identified microbial abundance QTLs at each level of microbial classification (e.g., family, genus) in fecal pellets and the cecal contents. We analyzed the composition of the gut microbiome to enumerate the microbial communities within these DO mice. We identified microbes associated with behavioral phenotypes using elastic net regularized regression and Permutation-based Maximum Covariance Analysis. To discover how microbes work together to influence behavior, microbes associated with the behavior were included as covariates in each behavior-specific conditional inference tree analysis, i.e., a tree-structured regression with unbiased covariate selection. A dashboard of interactive visualization modules enables the scientific community to interrogate and explore the microbe-microbe interactions and the microbial abundance QTL are also made accessible through an online QTL viewer. Finally, we have utilized CC mice and gene knock-out mutants to test the haplotype and gene effects of the loci on microbial abundance. Using multiple statistical approaches, we have identified numerous microbe and behavior associations. Together these results support the role of host genetics controlling the microbial abundance and microbial community composition being associated with behavior. U01DA043809 to GMW/JAB; P50DA039841to EJC

93 **Intestinal tumor proliferation requires and promotes wax ester production from hepatocyte-like cells through PDGF/VEGF signaling** Kerui Huang[1], Ting Miao[2], Ying Liu[2], Arpan Ghosh[3], Norbert Perrimon[2,4] [1]Genetics, Harvard Medical School, [2]Harvard Medical School, [3]Dyno Therapeutics, [4]Howard Hughes Medical Institute

The *Drosophila* tracheal system is a model to study angiogenesis as increased tracheal terminal branching is induced by gut

tumors that require oxygen for growth. We specifically induced an active oncogene *Yorkie* (*yki*^{3SA}), the homolog of human Yap1, to induce gut tumor formation. We discovered that tumor growth, in addition to increased tracheal terminal branching induced by gut tumors, required Hepatocyte nuclear factor 4 (*Hnf4*) in hepatocyte-like cells (oenocytes). *Hnf4* was activated by the target of rapamycin complex I (TORC1) in oenocytes. Specifically, over-expressing *Tsc1* and *Tsc2* (TORC1 inhibitors) or knockdown *Hnf4* in oenocytes blocked tumor-induced tracheation, reduced the rate of tumor growth, tumor induced intestinal stem cell (ISC) proliferation, alleviated tumor induced cachexia and increased lifespan. We identified a common downstream target of *Hnf4* and TORC1 specifically expressed in oenocytes, the fatty acid elongase *CG18609,* homologous to human ELOVL7. Interestingly, *CG186*09 is highly induced in flies with *yki* tumors and can be blocked by either *TSC1,2* induction or *Hnf4* knockdown in oenocytes. In addition, gut tumor growth induced the expression of the secreted ligand PDGF- and VEGF-related factor 1 (*Pvf1*), suggesting that Pvf1 from the gut non-autonomously regulates TORC1 and *Hnf4* activity in oenocytes – which is supported by an increase in *CG18609* expression when an active form of PDGF- and VEGF-receptor related (*PvR)* is expressed in oenocytes. Because Hnf4 in oenocytes is a major regulator of lipid metabolism, we performed lipidomic analysis and observed a dramatic increased level of Wax Esters (WEs) in the hemolymph circulation of *yki* flies. As over-expressing *TSC1,2* or *Hnf4* knockdown in oenocytes reduced the level of WEs during tumor formation, we propose that WEs are produced from oenocytes to assist tracheation. Finally, in non-tumor flies, either *TSC1,2* induction or *Hnf4* knockdown decreased tracheation in the midgut. Altogether, our study identifies a new paradigm where gut tumors non-autonomously induce the *Hnf4* activity in hepatocyte-like cells, increases the production of WEs that promote tracheation, which in turn promotes tumor growth.

94 **A novel antidiuretic hormone governs tumor-induced renal dysfunction** Wei Roc Song, Wenhao Xu, Gerui Li, Yuan Chen, Xujun Ye Medical Research Institute, Wuhan University

Maintenance of renal function and fluid transport is essential for both vertebrates and invertebrates to adapt to physiological and pathological challenges. It has been observed that subjects bearing malignant tumors frequently develop detrimental renal dysfunction and oliguria, with previous studies suggesting the involvement of chemotherapeutic toxicity and tumor-associated inflammation. However, the direct modulation of renal functions by tumors remains largely unclear. In this study, using conserved tumor models in *Drosophila,* we characterized isoform F of ion transport peptide (ITP_F) as the first fly antidiuretic hormone that is secreted by a subset of *yki*^{3SA}-gut tumor cells to impair renal function and cause severe abdomen bloating and fluid accumulation. Mechanistically, tumor-derived ITP_F targets the GPCR TkR99D in stellate cells (SCs) of renal-like Malpighian tubules (MTs), an excretory organ equivalent to renal tubules, to activate NOS/cGMP signaling and inhibit fluid excretion. We further uncovered previously unrecognized antidiuretic functions of mammalian neurokinin 3 receptor (NK3R), the homolog of fly TkR99D, as pharmaceutical blockade of NK3R efficiently alleviates renal tubular dysfunction in mice bearing different malignant tumors. Altogether, our results demonstrated a novel antidiuretic pathway mediating tumor-renal crosstalk across species and offered therapeutic opportunities for the treatment of cancer-associated renal dysfunction.

95 ***Drosophila* brain metastasis model uncovers injury-response-like cellular dynamics between host and tumor cells** Chaitali Khan, Nasser Rusan Cell and Developmental Biology Centre, NHLBI/National Institute of Health

The tumor microenvironment, constituted by resident and recruited cells, is critical in metastasis at secondary organ sites. Several studies have dissected the role of recruited immune and stromal cells in metastasis; however, the complexity of organs has limited our understanding of tissue-resident cells and heterogeneity among different cell types in supporting tumor metastasis. Simpler model organism like *Drosophila* provides a tractable system to investigate the function of tissue-resident cells in the context of whole organ and organism. However, to this date, very few *Drosophila* metastasis models exist. To overcome this, we developed a reliable model for studying metastatic tumors at the secondary organ sites by modifying the classic allograft transplantation assay. Our method demonstrates robust metastasis in adult *Drosophila* organs, including the brain and ovaries. We used this fly brain model first to investigate one of the least understood cancer phenomena: how a tumor invades the brain surface and breaches the blood-brain barrier (BBB). Our system allows unprecedented analysis of cellular dynamics of brain tumor invasion at different metastatic stages. Like mammals, fly brains contain a structure equivalent to the BBB, comprising two sets of surface glial cells, the perineurial glia (PNG) and the sub-perineurial glia (SPG). Further, extracellular matrix (ECM) proteins form the outermost layer of the brain - the basement membrane (BM). Our analysis of the early stages of invasion revealed that tumors remodeled the BM by mechanical forces driven by a collective mode of tumor invasion. We discovered that cells were remodeled yet remained associated with the moving invasive front. In addition, the tumor-glial interface at the invasive front contained disorganized ECM components, suggesting that matrix secretion might be essential for brain metastasis. Furthermore, the invasion sites recruited tumor-associated macrophages (TAMs) and exhibited selective upregulation of Jun-Kinase in BBB glial cells. Together, these results suggest invading tumors elicit a response reminiscent of a brain injury in surface glial cells, which helps maintain the invasive front and facilitates brain tumor invasion. The ongoing work investigates the functional requirement of responses generated by glial and recruited TAMs in brain tumor invasion. Thus, our study, for the first time, established a robust *in vivo* system to carry out cellular and

molecular genetic analysis of the metastatic invasion and colonization of the brain.

96 **A high sugar diet, but not obesity, reduces female fertility in *Drosophila melanogaster*** Rodrigo Dutra Nunes[1,2], Daniela Drummond-Barbosa[3,4] [1]Genetics Department, University of Wisconsin-Madison, [2]Morgridge Institute for Reasearch, [3]Genetics, University of Wisconsin-Madison, [4]Morgridge Institute for Research

Obesity is linked to reduced fertility in various species, from *Drosophila* to humans. Considering that obesity is often induced by changes in diet or eating behavior, it remains unclear whether obesity, diet, or both reduce fertility. In this study, we untangle these factors. We show that *Drosophila* females on a high sugar diet become rapidly obese and less fertile due to increased apoptotic death at two oogenesis stages: early germline cysts and vitellogenic egg chambers. They also have high glycogen, glucose, and trehalose levels and develop insulin resistance in their fat bodies (but not ovaries). By contrast, females with adult adipocyte-specific knockdown of anti-obesity genes that act through distinct mechanisms, *brummer* and *adipose*, are obese but have normal fertility. Remarkably, females on a high sugar diet supplemented with a separate source of water have near normal fertility and glucose levels, despite persistent obesity, high glycogen and trehalose levels, and fat body insulin resistance. These findings demonstrate that a high sugar diet affects specific processes in oogenesis independent of insulin resistance; that high glucose levels correlate with reduced fertility on a high sugar diet; and that obesity alone does not impair fertility. We are currently investigating the mechanisms underlying how a high sugar diet affects oogenesis using an unbiased, multi-organ proteomic approach. The functional screening of prioritized candidate genes will provide new insight into how a high sugar diet alters the communication among tissues to cause elevated glucose levels and decreased fertility.

97 **Sensing of dietary amino acids and metabolic regulation through Adipokinetic hormone-mediated brain-fat body axis in *Drosophila melanogaster* larvae** Muhammad Ahmad[1], Norbert Perrimon[1], He Li[2] [1]Genetics, Harvard Medical School, [2]University of Science and Technology of China

Perturbation of the balanced actions of insulin and glucagon can result in a number of physiological diseases. While sugar-mediated control of insulin and glucagon is well-established, little is known about the regulation of glucagon system by dietary proteins compared to insulin. Dietary amino acids promote the secretion of Drosophila Insulin-Like Peptides (Dilps) from the Insulin Producing Cells (IPCs). In this work, we sought to explore the possibility that Drosophila adipokinetic hormone (AKH), which is related to mammalian glucagon, is regulated by dietary amino acids. Using Ca2+-imaging and ex vivo culture of larval brains and fat, we show that secretion of AKH is under the control of specific dietary amino acids. We demonstrate that Akh-producing cells (APCs) in the larval brain directly sense the levels of extracellular amino acids and release AKH in response to changes in amino acid levels. Exposure of the larval brain to physiological concentrations of amino acids increases free cytosolic Ca2+ in AKH-producing neurons, stimulating them to release AKH. This, in turn, triggers waves of intercellular Ca2+ across the larval fat body. Such intercellular Ca2+ waves have also been observed in mammalian adipose tissue, where they have been proposed to have a metabolic pathway role. While dietary sugar and protein are known to positively regulate insulin, our study delineates that certain amino acids can regulate glucagon. This suggests that protein-mediated hormonal control of energy homeostasis is not simply centered around sugars and instead, includes sensing and control by additional nutritional factors.

98 **Vitamin B12 can rescue tissue-fusion mutant survival in *C. elegans* embryonic development by altering mitochondrial metabolism** Erin Hsiao[1], Tushar H Ganjawala[1], Prativa Amom[1], Radmehr Molaei[1], Alekhya Kondragunta[1], Amanda L Zacharias[2,3] [1]Developmental Biology, Cincinnati Children's Research Foundation, [2]Developmental Biology, Cincinnati Children's Research Foundation, [3]Pediatrics, University of CIncinnati College of Medicine

In the natural environment, *C. elegans* embryos develop robustly despite daily temperature shifts, different bacterial food sources, and other variable environmental conditions. In standard laboratory conditions, the worms receive a single food source, *E. coli*, resulting in partial deficiency of vitamin B12, which functions in one carbon metabolism (methionine/SAM cycle) and propionic acid breakdown in mitochondria. In humans, maternal deficiency in vitamin B12 can result in persistent neurological defects, including neural tube closure defects.

We investigated the impact of vitamin supplementation on mutants carrying mutations in the ephrin receptor gene, *vab-1*. Over half of *vab-1* mutant embryos fail to hatch due to defects in the closure of the ventral gastrulation cleft, a tissue fusion event similar to mammalian neural tube closure or palatal fusion. We found vitamin B12 supplementation partially rescues the embryonic lethality of seven different *vab-1* mutant strains, but does not affect neuronal and head morphogenesis phenotypes. The average embryonic hatch rate increased from 40% to 63% for null alleles across multiple days of egg laying. Feeding *Comamonas*, a bacteria which produces vitamin B12, results in a similar level of embryonic rescue. Vitamin B12 supplementation also partially rescued mutants carrying mutations in *vab-2, eph-2, eph-4* (all ephrin ligands), as well as the *sax-3*/Robo receptor, and the semaphorin pathway gene *mab-20*, indicating it affects other signaling pathways that promote ventral cleft closure. Disrupting levels of major B12 processing enzymes prevents vitamin B12 supplementation from

rescuing *vab-1* mutant embryos but disrupting levels of key enzymes in the methionine/SAM cycle did not, in contrast to its function in larval development (Watson et al., 2014). Instead, we found that key enzymes in the mitochondrial propionic acid pathway are required for vitamin B12 to rescue. This pathway converts a toxic byproduct to a source of energy. Consistent with a potential role of oxidative stress, antioxidants can also partially rescue Ephrin and Robo receptor mutants. We are currently investigating the hypothesis that vitamin B12 can partially rescue the tissue fusion event because increasing energy availability and reducing stress in migrating cells might make them more likely to complete the process despite suboptimal signaling. Our findings indicate that *C. elegans* has strong potential to investigate gene-metabolite interactions and study the role of metabolism in basic developmental processes.

99 **Biallelic Variants in *KMO* Cause a Novel Form of Congenital NAD Deficiency** Nathalie M Aceves[1], Chih-Wei Hsu[2], Nanbing Li-Villareal[2], Xiaohui Li[1], Seema Lalani[1,3], Jill A Rosenfeld[1], Angelina Gaspero[1], Denise Lanza[1], Audrey E Christiansen[2], Tara L Rasmussen[2], Mary E Dickinson[2], Brendan Lee[1,3], Ronit Marom[1,3], Undiagnosed Diseases Network[4], Hartmut Cuny[5,6], Sarah H Elsea[1], Sally Dunwoodie[5,6,7], BCM Center for Precision Medicine Models[8], Jason Heaney[1], Lindsay C Burrage[1,3] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Integrative Physiology, Baylor College of Medicine, [3]Texas Children's Hospital, [4]Undiagnosed Diseases Network, [5]Victor Chang Cardiac Research Institute, [6]School of Clinical Medicine, Faculty of Medicine and Health, University of New South Wales, [7]Faculty of Science, University of New South Wales, [8]Baylor College of Medicine

Deficiencies in enzymes within the kynurenine pathway (KP), which is responsible for synthesizing nicotinamide adenine dinucleotide (NAD+) from the amino acid tryptophan, have been associated with phenotypes that include congenital anomalies, recurrent miscarriages, and NAD deficiency. Biallelic variants in three genes within the KP, *KYNU*, *HAAO*, and *NADSYN1*, have been linked to congenital NAD deficiency disorder (CNDD). Through the Undiagnosed Diseases Network at Baylor College of Medicine (BCM), we identified an individual who inherited biallelic variants in kynurenine 3-monooxygenase *(KMO*), which encodes a KP enzyme. The patient presented with a complex phenotype characterized by short stature, multiple congenital anomalies, elevated levels of precursor metabolites, and low levels of NAD. Our working hypothesis is that *KMO* deficiency represents a novel form of CNDD and increases the risk of congenital anomalies. To test our hypothesis, we generated a knockout mouse model ($Kmo^{-/-}$) and confirmed the deletion with western blot and gene expression analysis. The $Kmo^{-/-}$ mice are viable and fertile when fed a breeder chow and display significantly increased serum kynurenine levels in contrast to their wild-type ($Kmo^{+/+}$) littermates. However, when the $Kmo^{-/-}$ mice are fed a niacin-deficient diet, they lose significant body weight, unlike their $Kmo^{+/+}$ counterparts. This failure to thrive is indicative of a functional deficiency in KMO, as the $Kmo^{-/-}$ mice are unable to efficiently produce NAD+ from tryptophan. Instead, they rely on NAD+ synthesized from niacin via the Preiss-Handler and salvage pathways. A similar genetic and environmental interaction was observed during embryogenesis. $Kmo^{-/-}$ embryos from $Kmo^{-/-}$ dams fed a low niacin diet have skeletal and vertebral anomalies, as well as a higher proportion of external congenital anomalies as compared to $Kmo^{+/-}$ littermates. Additional analyses using micro-computed tomography imaging to assess these embryos for internal soft-tissue abnormalities are ongoing. Overall, these $Kmo^{-/-}$ embryos exhibit phenotypes similar to those observed in human CNDD, such as shortened long bones, vertebral anomalies, and other congenital anomalies. Our ongoing studies aim to assess whether niacin supplementation can mitigate these phenotypes and may provide insight on this novel form of CNDD as well as the roles of NAD and gene-environment interactions during embryonic development.

100 **Drosophila Spaetzle, an Ortholog of Human Nerve Growth Factor–β, induces Squamous Cell Carcinoma** Saurabh Singh Parihar, Jyoti Tripathi, Pradip Sinha BSBE, Indian Institute of Technology, Kanpur

Two broad strategies help decipher the functional conservation of a human protein in *Drosophila*: by expressing the heterologous human protein or by ectopically expressing its *Drosophila* ortholog. Using the latter approach, here we show that Drosophila, Spaetzle, Spz—a distant functional ortholog of vertebrate Nerve Growth Factor-β (NGF-β)—induces squamous cell carcinoma (SCC) in *Drosophila* Male Accessory Gland (MAG), an organ equivalent to mammalian prostate. Thus, an ectopic gain of active-Spz induces SCC, as suggested by cell hypertrophy, disruption of cell cytoskeleton and polarity, increased invasion, and endoreplication. Interestingly, Spz-induced SCC is independent of the canonical Toll-1-mediated signaling. We further showed that the gain of Spz downregulates Yki-mediated signaling and upregulates JNK signaling in the MAG cells. Furthermore, a gain of Spz can cooperate with other oncogenes to induce cachexia and increase morbidity in adult hosts. Additionally, an increased level of Spz in circulation triggers a low-grade chronic renal inflammatory (CI), which in turn can induce nephrocyte dysfunction—an equivalent cell type to the mammalian podocytes. Our study, therefore, reveals a hitherto unrecognized functional orthology between *Drosophila* Spz and human NGF-β and also suggests a novel role of *Drosophila* neurotrophin signaling in carcinogenesis.

101 **Genetic and maternal determinants of adaptive tail length divergence in tropical and temperate house mice (*Mus musculus domesticus*)** Sylvia M Durkin, Michael W Nachman Museum of Vertebrate Zoology and Department of Integrative

Biology, University of California, Berkeley

Adaptive phenotypic variation may reflect a combination of genetic and environmental effects. Describing the relative roles of these effects is essential to understand how adaptive phenotypes evolve and persist in natural populations. However, this is difficult in systems where experimental manipulation is not feasible. Here, we use a combination of behavioral, morphological, and experimental genetic approaches to disentangle the genetic vs. maternal control of adaptive tail length divergence between tropical and temperate house mice. Tail length is longer in tropical mice, likely reflecting adaptation to warmer temperatures through increased extremity length (Allen's rule). This difference in length is due to both a greater number of vertebrae and to a greater length of vertebrae. First, we focused on the maternal environment, as this source of variation may be particularly important in mammals due to the obligate association between mother and offspring during gestation and lactation. Through cross-fostering experiments we show that tail length, but not body weight, is strongly influenced by the postnatal maternal environment. The maternal effect on tail length is likely mediated by differences in nest temperature due to differences in maternal behavior. Further, analysis of tail vertebrae variation in cross-fostered and pure line offspring suggests that the maternal environment influences tail vertebrae length, but not vertebrae number. In contrast, differences in the number of tail vertebrae between tropical and temperate mice appear to be genetically determined. Second, we took a QTL mapping approach to identify candidate genomic regions underlying divergent tail length and tail vertebrae number between tropical and temperate house mice. We measured tail length and counted the number of caudal vertebrae in a large F3 mapping panel of mice derived from crosses between temperate and tropical mice. These mapping studies identify genomic intervals containing genes with developmental mutants known to affect the number of caudal vertebrae, including *Hoxd13* and *Lin28a*. Together, these results provide a detailed account of how both maternal effects and genetic changes influence phenotypic divergence associated with adaptation to differing climates.

102 **Genetic changes underlying adaptation to CO2 in the Drosophila suzukii species complex** Alice Gadau[1], Xin Yu Zhu Jiang[2], Sasha Mills[3], Katherinee I Nagel[4], Li Zhao[5] [1]Zhao Lab, The Rockefeller University, [2]Hunter College, [3]The Rockefeller University, [4]The Nagel lab, New York University Langone, [5]Zhao lab, The Rockefeller University

Adaptation is fundamental to the survival and reproduction of living organisms. However, the genetic and neural basis underlying adaptation to new environments remain largely unknown. *Drosophila suzukii*, commonly known as the spotted wing fruit fly, has evolved to oviposit in ripe fruit. This novel behavior is unlike most *Drosophila* species, which prefer to oviposit in rotten fruit. The two other members in the *D. suzukii* subgroup—*D. biarmipes* and *D. subpulchrella*—show an intermediate preference for rotten fruit. Adaptive behavioral shifts have previously been correlated to changes in the coding sequence (CDS) of *Drosophila* sensory receptors. Morphological adaptation has been attributed to changes in *cis*-regulatory elements. However, the impact of *cis*-regulatory changes on sensory receptor retuning has never been explored. In this study, we focused on one of the CO_2 co-receptors, *Gr63a*, to investigate if adaptive behavioral shifts can be tuned by *cis*-regulatory factors. The CO_2 receptor offers a unique opportunity to address this question as it consistently responds to CO_2 across different Drosophila species, unlike other odorant receptors, which can have ligand shifts between species. Additionally, ripe fruit emits more CO_2 than rotten fruit, to which *D. suzukii* and *D. subpulchrella* may have adapted. We first found key differences in (1.) egg-laying preference in the presence of CO_2 using behavioral trials and (2.) sensitivity changes of the CO_2 neuron between *D. melanogaster*, *D. suzukii*, and *D. subpulchrella* using single sensillum electrophysiology (SSE). We then screened and found that *Gr63a* is under selection in *D. suzukii* and significantly highly expressed in *D. subpulchrella*. This suggests that two separate mechanisms may have evolved, changes in the CDS for *D. suzukii* and changes in the *cis*-regulatory element in *D. subpulchrella*. To test this hypothesis, we developed transgenic *D. melanogaster* flies that are expressing either the *D. melanogaster*, *D. subpulchrella* or *D. suzukii Gr63a cis*-regulatory factor or coding sequence in a common trans environment. We then used SSE, immunohistochemistry, and egg-laying behavior trials to understand how the CDS or *cis*-regulatory element can contribute to adaptive shifts, establishing a connection between genetic and behavioral modifications.

103 **Continuously fluctuating selection reveals extreme granularity and parallelism of adaptive tracking** Mark C Bitter[1], Skyler Berardi[2], Hayes Oken[2], Andy Huynh[1], Paul R. Schmidt[2], Dmitri Petrov[1] [1]Department of Biology, Stanford University, [2]Department of Biology, University of Pennsylvania

Temporally fluctuating environmental conditions are a ubiquitous feature of natural habitats. Yet, how finely natural populations adaptively track fluctuating selection pressures via shifts in standing genetic variation is unknown. We generated high-frequency, genome-wide allele frequency data from a genetically diverse population of *Drosophila melanogaster* in extensively replicated field mesocosms from late June to mid-December, a period of ~12 generations. Adaptation throughout the fundamental ecological phases of population expansion, peak density, and collapse was underpinned by extremely rapid, parallel changes in genomic variation across replicates. Yet, the dominant direction of selection fluctuated repeatedly, even within each of these ecological phases. Comparing patterns of allele frequency change to an independent dataset procured from the same experimental system demonstrated that the targets of selection are predictable across years. In concert,

our results reveal fitness-relevance of standing variation that is likely to be masked by inference approaches based on static population sampling, or insufficiently resolved time-series data. We propose such fine-scaled temporally fluctuating selection may be an important force maintaining functional genetic variation in natural populations and an important stochastic force affecting levels of standing genetic variation genome-wide.

104 **Recent population collapse shapes deleterious variation across subpopulations of the endangered Florida scrub-jay.** Mitchell G Lokey[1,2], Tram N Nguyen[3,4], Elissa J Cosgrove[1], Felix EG Beaudry[5], Nancy Chen[5], Reed Bowman[6], John W Fitzpatrick[4], Philipp W Messer[2], Andrew G Clark[1,2] [1]Molecular Biology & Genetics, Cornell University, [2]Computational Biology, Cornell University, [3]Ecology and Evolutionary Biology, Cornell University, [4]Lab of Ornithology, Cornell University, [5]Department of Biology, University of Rochester, [6]Archbold Biological Station

The "small population paradigm" of conservation genetics predicts that small and declining populations have an elevated risk of extinction due to: 1) weakened purifying selection leading to greater stochastic increases in the frequency of deleterious alleles, and 2) increased homozygosity of deleterious variants due to increased inbreeding. An exciting opportunity to investigate these predictions is in the endangered Florida scrub-jay (FSJ; *Aphelocoma coerulescens*) study system. As anthropogenic factors shrank Florida's scrub habitat over the last two centuries, the FSJ population subsequently declined to less than 10% of its former number. Spatially variable habitat and population decline in combination with the FSJ's non-migratory behavior led them to their current state of semi-isolated genetic metapopulations, with extirpation in some local areas. The diversity in size and stability among the remaining FSJ subpopulations allows us to study the effects of recent decline, fragmentation, small size, and management on genetic variation within a single species. Here we explore the effects of these factors on inferred deleterious variation using 21x whole-genome sequencing of 241 birds sampled from 5 demographically diverse subpopulations over two time points. To identify putatively-deleterious variants (PDVs) we apply gene prediction and conservation-based approaches. We then quantify the impacts of small and declining population size on the efficacy of purifying selection by contrasting the site frequency spectra of PDVs and neutral variation across subpopulations as well as the masked and realized burden of PDVs across individuals. We find that the smaller subpopulations show increased PDV allele frequencies and clearly elevated genetic load, in terms of both the realized and masked load. Finally, we use forward-genetic simulations to 1) explore the evolution of genetic load for subpopulations of different sizes during the demographic history of the FSJ, 2) assess whether the effects of demographic processes are consistent with observed variation, and 3) predict each subpopulation's future trajectory of genetic load.

105 **Gene amplification as a driver of insect adaptation to transgenic crops: The case of *Helicoverpa zea*** Katherine L Taylor, Jane Quackenbush, Cara Lamberty, Kelly A Hamby, Megan Fritz University of Maryland

The evolution of insect resistance to agricultural management practices is a long-standing problem in food production systems, which results in crop failure and economic losses. Historically, most cases of field-evolved insect resistance have been attributed to *de novo* mutations of large effect at a single locus. As a result, many U.S. agricultural policies and practices for insect resistance management have been shaped under assumptions of single locus resistance evolution. The phytophagous North American insect pest, *Helicoverpa zea*, has recently evolved high levels of resistance to corn and cotton expressing transgenes from the bacterium *Bacillus thuringiensis* (Bt). Our work has examined the genome architecture of field-evolved Bt resistance in *H. zea* using whole genome sequencing, differential gene expression analysis, and functional assays. We show that adaptation to Bt crops by *H. zea* does not result from *de novo* mutation at a single locus, but is the result of multi-locus selection on standing genetic variation across the genome. Interestingly, one moderate effect locus includes a cluster of 7 paralogous genes, and up to 5 copies of this gene cluster are present in resistant *H. zea* populations. Our findings provide empirical evidence that evolutionarily novel resistance traits can arise as a result of polygenic adaptation from standing genetic variation. Moreover, they suggest that a paradigm shift in policy development is needed to more effectively detect and delay resistance evolution in agricultural ecosystems.

106 **Comparative genomics for California's wildlife: linking estimated migration rates and population demographics** Erik Enbody[1], John Novembre[2], Russ Corbett-Detig[1] [1]UC Santa Cruz, [2]University of Chicago

Population genomic methods to infer population structure and health are commonly employed in diverse datasets, but the results are rarely compared among taxa. Such multi-taxa comparisons on the geographical structure of populations have broad implications for understanding how the landscape shapes community dynamics while mitigating the idiosyncratic effects of individual taxa. In conservation genomics, most studies to date are single-taxa, but a general application to decision-making and land purchasing requires integrating genomic data across entire communities. Here, we take advantage of a massive taxonomically diverse population genomic dataset comprising over 150 species (nearly 20,000 samples) sampled densely across a uniform landscape as part of the California Conservation Genomics Project. This presents a unique opportunity to ask how the landscape impacts population genetic change across an entire community. Existing single-taxon methods have

successfully applied circuit theory to visualize geographic structure using maps of varying migration rates that can explain the observed genotype frequency among geographically clustered samples. We will share an overview of a methodology for comparing effective migration surfaces across different species on a uniform landscape. We recover major biogeographic breaks, common across as diverse taxa as birds, mammals, and insects, and examine taxa with properties that show different patterns. We ask how migration histories influence population health, such as inbreeding history, and the impact of life history characteristics on these patterns. Together, our methodology provides a powerful framework for future large-scale landscape and conservation genomics projects interested in understanding the landscape genomics of entire communities.

107 **Systematic mapping of natural variants that bypass essential genes** Amandine Batté[1], Núria Bosch[1], Carles Pons[2], Marina Ota[1], Maykel Lopes[1], Jolanda van Leeuwen[1] [1]Center for Integrative Genomics, University of Lausanne, [2]IRB Barcelona

Mutations often show phenotypic differences across genetically distinct individuals. In the most extreme case, a gene can be essential in one genetic background but have no effect on viability in another. Here, we investigated the frequency and underlying causes of differences in gene essentiality across 19 genetically diverse natural yeast strains. We identified 68 context-dependent essential genes that were not required for viability in at least one genetic background, suggesting that the strain contained variants that could bypass the requirement for the essential gene. Although some genes appeared to be essential in the reference background only, the vast majority of genes were nonessential in only one or two genetic backgrounds. We identified and validated the causal bypass suppressor variants for all 68 context-dependent essential genes using bulk segregant analysis and allele replacements. Bypass suppression was generally driven by a single, strong modifier gene, and involved both genes directly counteracting the molecular defect caused by loss of the context-dependent essential gene, as well as general modifiers bypassing the requirement of many genes. For example, a variant in the karyopherin *MSN5* could bypass the essentiality of the checkpoint kinase *RAD53* by modulating histone levels. Our findings highlight the frequency of genetic background effects and range of underlying mechanisms, providing insight on how genetic variance accumulates during evolution and affects genetic traits.

108 **Epigenetic Silencing of Transposable Elements Drives Variation In Recombination Landscapes** Yuheng Huang[1], Zita Y Gao[1], Kevin HC Wei[2], Grace Yuh Chwen Lee[1] [1]UC-Irvine, [2]UBC

Meiotic recombination is a prominent force shaping genome evolution, and understanding the causes for varying recombination landscapes within and between species has remained a central, though challenging, question in the field of evolutionary genomics. Across species, chromosome-wide recombination maps have shown strongly suppressed rates of recombination around pericentromeric heterochromatin, and the enrichment of repressive epigenetic marks in this genome compartment has been attributed as the cause. Interestingly, in the euchromatic regions of the genome, repressive epigenetic marks are also found enriched at transposable elements (TEs), widespread genomic parasites, as a consequence of the host-directed mechanism to epigenetically silence TEs. Building upon these observations, we hypothesize that the epigenetic silencing of polymorphic TEs in the euchromatic genome could locally suppress recombination, driving varying recombination landscapes between individuals. To test our hypothesis, we studied the associations between the distribution of crossover events, one of the two important recombination products, and epigenetically silenced TEs in two *Drosophila* strains with distinct TE insertion profiles. We developed a novel approach that uses PacBio long-read sequencing to efficiently identify crossover events among a large number of pooled recombinant individuals. According to our benchmark data, our approach has a low false positive rate (6.5%) and randomly distributed false-negative events. For each strain, we identified ~1,400 crossover events in a single PacBio sequencing reaction, with a high resolution of crossover breakpoints (within 1kb). Supporting our hypothesis, we found that TEs, mainly those leading to local enrichment of repressive epigenetic marks, negatively impacted crossover rates. Such an effect contributes to varying crossover maps between individuals of the same species. By performing forward population genetic simulations, we uncovered that this suppressive effect on recombination could further drive the evolution of enhanced epigenetic silencing of TEs. Overall, our investigations reveal how the selfish genetic elements could be active modifiers of the recombination landscapes through epigenetic mechanisms, shaping genome evolution within and between species.

109 **Temporal Dynamics of Phase Transitions: Transcriptome Profiling in Swarming Locusts** Maeva A. Techer, Alyssa Canova, Vivian A. Peralta Santana, Hojun Song Entomology, Texas A&M University

Density-dependent polyphenism has evolved multiple times in insect taxa, but locusts often embody this phenomenon (Orthoptera: *Acrididae*). In response to conspecific population density changes, solitary, shy and cryptic grasshoppers can develop into gregarious, swarming, and conspicuous locusts (i.e., gregarization); or revert (i.e., solitarization). Extensive research on the mechanical and physiological aspects of these phase transitions has focused on the swarming desert locust (*Schistocerca gregaria*), one of the 19 recognized locust species. Despite differing temporal dynamics in gregarization and solitarization in desert locusts, both processes are triggered by a sensory cues, including sight, smell, and tactile stimulation.

These mechanosensory-induced developmental changes are accompanied by significant gene expression changes in nervous tissues following the phase change. Yet, the intricate molecular mechanisms underlying the early stages of locust phase transition remains unknown.

We aim to unravel the temporal gene expression changes and regulatory networks underlying phase transitions in lab-reared desert locusts. To induce gregarization, solitarious nymphs isolated for two generations were exposed to crowded cages with high conspecific densities (> 300 individuals) for durations ranging from 30 minutes to 72 hours. Conversely, to induce solitarization, long-term gregarious nymphs were isolated in sensory-independent cages. At each time point, we collected and sequenced various nervous and chemosensory tissues involved in environmental cue processing. In this talk, we will share insights from 520 tissue-specific transcriptomes sequenced using Illumina short-reads. The time course transcriptomic analysis was guided by the six new reference chromosome-length *Schistocerca* genomes (~8.5Gb) developed by the BPRI and USDA. Ultimately, we will sequence and compare the temporal transcriptomic landscapes of six *Schistocerca* species displaying varying degrees of density-dependent phenotypic plasticity to pinpoint the molecular origins of locust swarming.

110 **A Telomere-to-Telomere (T2T) complete mouse genome** Thomas Keane[1], Bailey Francis[1], Mohab Helmy[1], Kevin Costello[2], Jingtao Lilue[3], Laura Reinholdt[4], Anne Czechanski[4], Emma Betterbridge[5], Iraad Bronner[1], Anne Ferguson-Smith[2], David J Adams[5] [1]EMBL-EBI, [2]Department of Genetics, University of Cambridge, [3]Oujiang Laboratory (Zhejiang Lab for Regenerative Medicine, Vision and Brain Health), [4]Jackson Laboratory, [5]Wellcome Sanger Institute

The generation and assembly of a reference genome for C57BL/6J revolutionized our ability to relate sequence to function. Despite over twenty years of effort, the current mouse reference genome (GRCm39) has over 170 known gaps and unresolved issues. Many important loci such as the centromeres, major histocompatibility complex (MHC) on Chromosome 17, the pseudo-autosomal region (PAR), and Krüppel-associated box (KRAB) domain-containing zinc-finger protein (KZFP) loci on Chromosome 2 and 4, remain incomplete or inaccurate. By using a combination of long and ultra-long read sequencing technologies, we have generated the most complete mouse reference genome for two strains from a C57BL/6J x CAST/EiJ F1 animal. Through a combination of automated genome assembly and manual curation, all of the C57BL/6J and CAST/EiJ autosomes are full length, which represents a major milestone in the journey towards a fully complete mouse reference genome. We have added approximately 240 Mbp of novel sequence compared to GRCm39, largely consisting of satellite sequence in the centromeres and telomeres. We have fully sequenced all but seven of the remaining autosomal gaps in GRCm39, which added approximately 12.7 Mbp of additional sequence containing tens of predicted protein coding genes. We find that telomeres are consistently longer in the C57BL/6J strain compared to CAST/EiJ, whilst telomere length varies dramatically between chromosomes. We compared the architecture of the PAR region between *Mus m. molossinus*, *Mus m. domesticus*, and *Mus m. castaneus*, identifying a larger PAR in *Mus m. castaneus* due to a multi copy expansion of the *Erdr1* gene. Finally, we have built the first complete picture of chromosomal inversions between two inbred mouse strains, finding five inversions larger than a megabase. Not only do our assemblies unlock some of the most challenging loci in the genome, but with the completed genomic sequences for two mouse strains, our work enables comparative analyses in these complex regions for the very first time.

111 **Convergent and lineage-specific genomic changes contribute to adaptations in nectar-taking birds** Ekaterina Osipova[1], Meng-Ching Ko[2], Maude Baldwin[2], Michael Hiller[3], Timothy Sackton[1] [1]Harvard University, [2]Max Planck Institute for Biological Intelligence, [3]Senckenberg Research Institute

Understanding the genomic basis of dietary adaptations can provide insights into the evolution of metabolic processes as well as into the extent and degree of convergent evolution. Remarkably, although high-sugar diets are associated with metabolic diseases in humans, several bird lineages have independently evolved to primarily subsist on simple sugars from flower nectar or fruits. In this study, we address a key question regarding the repeatability of molecular evolution by investigating the convergent and lineage-specific molecular mechanisms underlying dietary adaptations in four major nectar-feeding bird lineages: hummingbirds, parrots, honeyeaters, and sunbirds. We generated ten high-quality genomic assemblies for nectar-feeding species and their closely related non-nectar-dependent counterparts, and obtained tissue-specific transcriptomes for six key species. Our comprehensive genome-wide investigation, encompassing both protein-coding and non-coding regulatory sequences, reveals evidence of adaptive evolution in processes related to glycolysis, blood pressure regulation, and heart function across all four nectar-feeding groups. Importantly, these convergent signals of selection may manifest at various levels - from pathways to individual genes and regulatory elements, highlighting the interplay of different types of changes in altering or regulating metabolic pathways. Furthermore, although we find that individual genes are rarely targeted in all lineages, our integrative analysis pinpoints key candidate genes, such as MLXIPL, that is vital for carbohydrate and lipid metabolism and which exhibits repeated signals of selection and shifts in expression levels across nectar-feeding lineages; functional assays demonstrate changes in nectarivores corresponding to these selection signals. Our findings suggest that both convergent and lineage-specific strategies have played roles in the evolution of nectar-feeding adaptations and underscore the complex

patterns of molecular convergence in both protein-coding loci and regulatory elements in shaping metabolic pathways.

112 **Evolutionary invention of a thermocouple-like sensor by adaptation of cytochrome c oxidase in a subterrestrial metazoan** TreVaughn S Ellis[1], Megan N Guerin[1], Mark J Ware[1], Alexandra S Manning[1], Ariana S Coley[1], Ali Amini[2], George C Chung[3], Kristin C Gunsalus[3], John S Bracht[1] [1]Biology, American University, [2]Data Science, American University, [3]Ceneter for Genomics and Systems Biology, NYU

In this study we report a naturally evolved temperature-sensing electrical regulator in the cytochrome c oxidase of the Devil Worm, *Halicephalobus mephisto*. This extremophile metazoan was isolated 1.3 km underground in a South African goldmine, where it adapted to heat and potentially to hypoxia, making its mitochondrial sequence a likely target of adaptational change. Here we report the full mitochondrial genome sequence of this organism, and show through dN/dS analysis statistically robust evidence of positive selection in *H. mephisto* cytochrome c oxidase subunits. Seventeen of these positively-selected amino acid substitutions were localized in proximity to the H- and K-pathway proton channels of the complex. Direct experimental measurement of the mitochondrial proton gradient confirmed that proton translocation is altered in *H. mephisto* relative to *C. elegans*. The *H. mephisto* cytochrome c oxidase proton pump completely shuts down at low temperatures leading to approximately five-fold reduction in the transmembrane proton gradient voltage (ΔΨm) when comparing 37°C with 20°C. Correspondingly, the lifecycle of *H. mephisto* slows by the same amount, becoming five-fold longer at the low temperature. By this simple mechanism the organism can sense the environmental temperature and adjust its growth rate correspondingly. A thermocouple device senses temperature fluctuations by voltage changes. Because the cytochrome c oxidase of *H. mephisto* has evolved to regulate its mitochondrial proton gradient voltage (ΔΨm) by temperature, it represents the first known example of a naturally evolved biological thermocouple-type device in the mitochondrion of any organism.

113 **Metabolic profile of Drosophila exercise-trained flies suggests NRF2 pathway as a link between exercise & oxidative stress** Tolulope R Kolapo[1], Miled A Maisonet-Nieves[2], Laura K Reed[2] [1]Biological Sciences, The University of Alabama, [2]Biological Sciences, University of Alabama

Metabolic Syndrome (MetS) is a cluster of several disorders that includes obesity, type II diabetes, and cardiovascular diseases. Various exercise-related studies in humans have identified the ameliorating effects of exercise on MetS. However, human experimental studies face several limitations due to the small population size, expensive sampling procedures, & uncontrollable environmental factors. *Drosophila melanogaster* is a suitable model to mimic various disease-related studies as seen in humans. The negative geotaxis tendency of flies has been studied to develop devices to exercise flies. The Power Tower and TreadWheel are devices used to study large sample sizes of fruit flies to determine the effects of exercise. However, controversies exist on the relatedness of fly exercise to human exercise. This is because indicators like physiological conditions, muscle biopsies, blood lactate, and V02 max cannot be measured *in situ* while exercise is ongoing in flies as it can in humans. To further determine the close relatedness of fly exercise research to humans, we conducted an untargeted metabolomics analysis of exercise-trained fruit flies and their controls after a five-day exercise regime. We also trained flies on the Power Tower and the TreadWheel to understand the devices effect on metabolic profiles of flies. Correlating device effects to metabolic profile could aid the classification of exercise devices for endurance, strength and resistance training as observed in human exercise research. Our result reveals close relatedness of fly exercise outcomes and human exercise outcomes at the metabolite levels. We identified increased urea, TCA cycle substrates such as aconitic acid and citric acid, and increased levels of protein synthesis, which are all exercise indicators. Also, we observed an upregulation of exercise-induced oxidative stress indicators and the induction of the *NRF2* pathway that regulates oxidative stress and antioxidant production. To further confirm the induction of oxidative stress we performed a gene expression study of six oxidative stress-related genes. Increased expression levels of these genes further suggest exercise-induced oxidative stress in trained flies. The downregulation of the *Gagr* gene suggests a localization of stress induced in the mitochondria. Through this study we confirmed that exercise activates pathways of oxidative stress in *Drosophila* and similarities exist in the metabolome of exercised fruit flies and humans.

114 **Pangenome analysis of 1,098 *Saccharomyces cerevisiae* isolates reveals the hidden genomic and genetic basis behind the phenotypic variation** Victor Loegler[1], Elie Teyssonniere[1], Pia Thiele[1], Andreas Tsouris[1], Jing Hou[1], Anne Friedrich[1], Joseph Schacherer[1,2] [1]Université de Strasbourg, CNRS, GMGM UMR 7156, Strasbourg, France., [2]Institut Universitaire de France (IUF), Paris, France.

Pangenomes from large natural population allow to capture the exhaustive genomic diversity and explore more deeply the cause of the missing heritability of complex traits. Identifying at a population-scale all type of genetic variants and measuring their phenotypic contribution is now essential. In this context, we sequenced and *de novo* assembled 1,409 chromosome-level genomes using a long-read sequencing strategy for more than a thousand natural isolates of *Saccharomyces cerevisiae* yeast, coming from diverse ecological and geographical origins. This dataset led to the detection and definition of an exhaustive

catalog of structural variants (SVs) present in yeast, highlighting adaptative and clade-specific SVs. Interestingly, our results showed different evolutionary trajectories in terms of accumulated mutational events, with wild clades having an enrichment in single nucleotide polymorphisms (SNPs), whereas some domesticated clades are enriched in SVs. We also constructed a high-resolution pangenome, showing a significant variation of the accessory genome at the species level. The pangenome clearly revealed signatures in terms of gene content related to specific populations. Using phenotypic data generated on this large population (fitness, transcriptomes and proteomes), this pangenome was then used for genome-wide association analyses to generate a detailed map of loci involved in the variation of fitness (QTL), transcript (eQTL) as well as protein abundance (pQTL). Increasing the number and type of genetic variants, such as SVs, involved in association analysis improves the power to identify causal factors and decreases the missing heritability. Finally, the data we generated represents a unique resource for the development of graph-based pangenome methods. To our knowledge, our study constitutes the most comprehensive study of genetic content and structural variations combined with their effect on phenotypic variation in any eukaryotic species.

115 **Unexpected roles for the bud site selection machinery in the establishment and maintenance of polarity in *S. cerevisiae* spores** Benjamin Cooperman, MICHAEL MCMURRAY Cell and Developmental Biology, University of Colorado Anschutz Medical Campus

Many mechanisms controlling the establishment and maintenance of eukaryotic cellular polarity were first discovered in *S. cerevisiae*. In mitotically dividing (vegetative) yeast cells, bud site selection both requires and directs the placement of stable landmark proteins that direct future bud sites. Landmark proteins are integral to the membrane and anchored to the cell wall. Landmarks interact with the cytoplasmic polarity machinery through the Ras-family GTPase Rsr1 module that recruits and activates Cdc42. Polarity landmarks can be overridden by mating pheromone, which redirects secretion toward the pheromone source. Sporulation is different: Newly made double-bilayer membranes grow from the meiotic outer plaques of the spindle pole bodies and engulf the haploid nuclei produced by meiosis. Following membrane closure, the outer bilayer is lost as a specialized cell wall is built between the bilayers. The spores remain encased in the ascus, the original wall of the diploid cell. It was thought that these newly made cells lack polarity landmarks and that germinating spores break symmetry when they bud or mate.

We previously discovered that, even before germination, *S. cerevisiae* spores are polarized in a manner that promotes polarized growth during germination away from the points of spore-spore contact. Consequently, if a spore buds, the bud penetrates the ascus. If sister spores mate, they do so only after first elongating away from each other, then repolarizing toward each other, creating a zygote with a distinctive morphology. Thus mating by sister spores involves a predetermined polarity program that initially overrides pheromones.

The septin Cdc10 was the only known constituent of the spore polarity site and the assembly mechanism was entirely unknown. We have now determined that the polarity site also contains the landmark protein Bud8 and Bud5, a soluble GEF for Rsr1. Exocyst subunits and the Myo2 motor protein arrive at the polarity site after spore membrane closure, followed by Bud8, which persists for months at a single site. The arrival of Myo2 before Bud8, and Myo2 departure afterwards, suggests that Bud8 is actively delivered via actin cables. We find that the Rsr1 module is dispensable for polarity site assembly, but is required for its use as a bud site. Our data support a model in which polarized trafficking to the meiotic outer plaque ultimately deposits Bud8 in the membrane, where it acts as a stable landmark to direct polarization upon germination. Because trafficking occurs at spindle poles, anaphase II pushes the sites to the ascus periphery. In addition to ensuring that buds are not trapped inside the ascus, polarized growth in these directions may sensitize pheromone detection by positioning receptors away from mixed, highly concentrated signals in the ascus center. Our work reveals unanticipated roles for the canonical polarity machinery in a previously unexplored context.

116 **The Role of Hemocytes in Cell Debris Clearance in the *Drosophila* Ovary** Alexandra Chasse[1], Kim McCall[1], Shruthi Bandyadka[2] [1]Biology, Boston University, [2]Boston University

Cell death is an important process in the body, as it occurs throughout every tissue in the body during different development, disease, and tissue regeneration. However, when a cell dies, it must be cleared away or the persistence of the corpse can be damaging to the organism. Phagocytes are responsible for clearing away these dying cells and are typically characterized as either professional or nonprofessional phagocytes. Professional phagocytes, such as macrophages, are found in nearly every part of the body while nonprofessional phagocytes, such as epithelial cells, are found in every tissue type. However, there are organs that are considered “immune-privileged” as they have little to no immune surveillance and rely on nonprofessional phagocytes to engulf dying cells. These organs are surrounded by barriers to protect the tissue from viruses, bacteria, and perhaps even immune cells. The Drosophila ovary is considered immune-privileged, however the presence of hemocytes, the macrophages of Drosophila, around the ovary suggests they may have a potential function. Using several fluorescent and

genetic tools, we were able to identify and analyze hemocyte localization and potential functions in response to starvation-induced cell death in the ovary. Using RNAi, hemocytes were genetically ablated and it has been determined that the presence of hemocytes affects oogenesis and that they phagocytose ovarian cell debris. Signaling mechanisms between hemocytes and follicle cells are currently under investigation. Since the mechanisms of cell death and clearance are highly conserved, these experiments will increase our knowledge of immunosurveillance of immune-privileged tissues and cross-talk between professional and non-professional phagocytes. Since the mechanisms of cell death and clearance are highly conserved, these experiments will increase our knowledge of immune surveillance of the ovary. It is likely that these findings will have implications in understanding and treating human disease, development, and fertility.

117 **From Drosophila to a human immunodeficiency and enhanced cancer cell immunotherapy: hyperactive Rac stimulates cellular cannibalism** Abhinava K. Mishra, Melanie Rodriguez, Lauren Penfield, Morgan Smith, Anthony Rodriguez, Meghan Morrissey, Denise J. Montell Molecular, Cellular, and Developmental Biology, University of California Santa Barbara

The small GTPase Rac is an essential regulator of cell shape and migration. Here we show that in Drosophila, hyperactivating Rac (Rac^{G12V}) stimulates ovarian follicle cells to cannibalize neighboring cells, destroying the tissue. Hyperactive Rac is sufficient to drive three different types of whole cell engulfment and/or killing. Rac^{G12V} expression in border cells is sufficient to cause them to engulf but not kill whole, living polar cells. In contrast, Rac^{G12V} expression in individual epithelial follicle cells induces caspase-dependent apoptosis in neighboring follicle cells and leads to engulfment. Border cells expressing Rac^{G12V} can also kill the entire germline in a process that begins with trogocytosis (nibbling) of the most anterior nurse cell followed by rapid and nearly synchronous non-apoptotic germ cell death.

In mammals, Rac2 is predominantly expressed in hematopoietic cells where it is essential for survival and motility. The hyperactivating mutation $Rac2^{E62K}$ also causes a human immunodeficiency, although the mechanism is unknown. Taking cues from the fundamental cell biology in the fruit fly ovary, we show that hyperactive $Rac2^{E62K}$ stimulates human HL60-derived macrophage-like cells to engulf and kill living T cell leukemia cells. Primary mouse $Rac2^{+/E62K}$ bone-marrow-derived macrophages (BMDMs) also cannibalize primary $Rac2^{+/E62K}$ T cells due to a combination of macrophage hyperactivity and T cell hypersensitivity to engulfment. Additionally, bone-marrow-derived $Rac2^{+/E62K}$ macrophages non-autonomously stimulate wild type macrophages to engulf T cells. $Rac2^{E62K}$ also enhances engulfment of target cancer cells by chimeric antigen receptor-expressing macrophages (CAR-M). We propose that Rac-mediated cell cannibalism may contribute to $Rac2^{+/E62K}$ human immunodeficiency and enhance CAR-M cancer immunotherapy.

118 **RAS and PLK-1 signaling intersect to control nuclear envelope dynamics in early embryogenesis** Han Bit Baek[1], Debabrata Das[2], Hongyuan Li[2], Shin-Yu Chen[2], Swathi Arur[2] [1]UT MD Anderson Cancer Center, [2]MD Anderson Cancer Center

Rat Sarcoma (RAS) is a small GTPase that transmits extracellular growth factor signals through a downstream kinase cascade to regulate genes that promote proliferation, differentiation, and survival. Constitutively active mutations in RAS promote tumor onset and metastasis, and germline mutations are associated with birth defects in humans. However, it is unclear if activating mutations in RAS drive both cancer progression and birth defects through similar mechanisms. RAS L19F is an activating mutation in the RAS GTPase domain and is found in human cancers. I used *C. elegans* to track the impact of this activating RAS mutation on embryonic development. In *C. elegans*, I discovered that the oncogenic RAS mutation inhibits nuclear envelope breakdown (NEBD) in the 1-cell embryo. Loss of NEBD at this stage impedes the fusion of the oocyte and sperm nucleus. This failure to merge the maternal and paternal nucleus in the 1-cell embryo blocked the formation of the zygotic nucleus, resulting in nuclear defects and impaired embryonic development. Strikingly, I found that RAS functions through Polo like kinase 1 (PLK-1), a master regulator of nuclear envelope dynamics across cell types and species. Specifically, I uncovered a direct regulation of PLK-1 by ERK (Extracellular signal-regulated kinase), the final downstream effector of RAS signaling wherein ERK phosphorylates PLK-1. To assess the *in vivo* outcomes of this phosphorylation, I mapped the ERK dependent PLK-1 phosphorylation sites and generated a phospho-null PLK-1 allele in *C. elegans*. The phospho-null PLK-1 allele in the oncogenic RAS background resulted in a significant rescue of the nuclear defects. Thus, I propose that RAS-ERK signaling directly regulates PLK-1 to control NEBD during development. This is the first observation that an oncogenic RAS mutation can lead to defects in NEBD and the first reported intersection between RAS signaling and PLK-1 activity, setting the stage to uncover a novel role of RAS during cell division.

119 **Aneuploidy-induced defects in Ribosome Quality Control disrupt quiescence and aging in wild *S. cerevisiae*** Leah E. Escalante[1], James Hose[2,3], Hollis Howe[3,3], Norah Paulsen[3,4], Audrey P. Gasch[2] [1]Center for Genomic Science Innovation, Univ Wisconsin-Madison, [2]Genetics, Univ Wisconsin-Madison, [3]Univ Wisconsin-Madison, [4]CGSI, Univ Wisconsin-Madison

Karyotype imbalance due to an abnormal number of chromosomes is deleterious during development, for reasons that remain unclear. In humans, Trisomy 21 that causes Down syndrome leads to myriad defects in development, stem cell

maintenance and renewal, and aging including premature neurodegeneration and shortened lifespan. However, the molecular causes remain murky despite decades of study. In the course of studying aneuploidy determinants in a wild-yeast model, we discovered that chromosome duplication in yeast causes phenotypes related to those seen in Down syndrome, including defects in nutrient-dependent cell-cycle arrest and quiescence entry along with shortened lifespan. These defects are independent of which chromosome is duplicated and observed across multiple wild-strain backgrounds. Remarkably, we discovered that cell cycle and quiescence defects are caused in part by defects in Ribosome Quality Control (RQC) upon chromosome amplification. Aneuploid cells show increased ribosome stalling on an RQC reporter, and genetic analysis confirms involvement of the RQC pathway. Aneuploidy-dependent defects can be alleviated by overexpressing limiting RQC subunits, including Rqc1 and E3 ubiquitin ligase Ltn1 (whose mammalian ortholog is implicated in neurodegeneration), or deletion of CATylating enzyme Rqc2 that can produce protein aggregates. In contrast, inducing ribosome stalling in euploids mimics aneuploid defects. We show that genetic perturbation of ubiquitin metabolism can either alleviate or exacerbate aneuploid defects. Our working model is that the accumulation of ubiquitinated RQC intermediates depletes free ubiquitin, disrupting turnover of cell-cycle regulators and other proteins while producing protein aggregates that disturb healthy physiology. These results expand our understanding of the mechanisms of aneuploidy toxicity and raise important implications for Down syndrome as well as aneuploid cancers.

120 ***Getting there in one piece:*** **The Rac pathway is required to maintain cellular integrity during long distance leader-cell migration in *C. elegans* gonadogenesis.** Noor Singh, Kacy L. Gordon Biology, University of North Carolina at Chapel Hill

In larval *C. elegans* hermaphrodites, the long-distance migration of two leader cells, the distal tip cells (DTCs), drives the morphogenesis of two gonad arms that make up the reproductive system. Each DTC also serves as a stem cell niche that maintains a continuously proliferating germline stem cell population in the distal gonad. Loss of the Rho GTPase CED-10 (ortholog of human Rac1) or any component of its activating GEF module (composed of CED-5/DOCK180, CED-12/ELMO, and CED-2/CrkII) has been shown to lead to DTC migration defects and aberrant gonad morphology through an unknown mechanism. Rac1 typically functions at the leading edge of protrusively migrating cells to drive cytoskeletal rearrangements and the formation of dynamic protrusions, but the DTC has been shown to migrate non-protrusively. With the use of genetic mutants, tissue specific and whole body gene knockdown, and live confocal imaging of cells and endogenously tagged proteins, we show that loss of these *ced* genes results in fragmentation of the DTC as it migrates. Both products of fragmentation—the now-smaller DTC and the membranous patch of cellular material—localize important stem cell niche signaling, migration, and adhesion factors to their surfaces. However, only one retains the DTC nucleus and the ability to maintain gene expression and new protein synthesis over time. Strikingly, this enucleate patch can lead a branch bifurcating off the gonad arm that grows through germ cell proliferation driven by aberrant LAG-2/Delta signaling to germ cells adjacent to the patch, mimicking the stem-cell niche activity of the DTC. However, germ cells in this branch differentiate as the patch loses LAG-2 signaling (the stemness cue) in adulthood. These results drive the conclusion that although the DTC does not undergo lamellipodial-driven migration, it still relies on cytoskeletal regulation under the control of the small GTPase, Rac1, to retain its integrity, shape and position at the tip of the growing gonad. This work also adds insights to DTC migration as a model for migration events in other organisms that are led by smooth edged leader cells, such as branching morphogenesis during the development of tubular organs in vertebrates.

121 **Actin filament stiffness regulates the rate of embryonic wound closure in *Drosophila*** Ji Hong R Sayo, Ana Maria Carmo, Rodrigo Fernandez-Gonzalez Institute of Biomedical Engineering, University of Toronto

Mechanical forces drive key cell behaviours for embryonic development, tissue repair, and metastasis. Force generation is often associated with a protein network formed by actin and the molecular motor non-muscle myosin II . During embryonic wound healing, the cells surrounding the wound preferentially localize actin and myosin at the wound edge, forming a supracellular cable around the wound that contracts to guide cell movements. But force generation can also occur in the absence of myosin motor activity, for example during cytokinesis, through a combination of actin filament severing and crosslinking. Whether motorless contraction contributes to embryonic wound closure is unknown. We are investigating the role of the actin-severing protein cofilin during *Drosophila* embryonic wound healing. We found that cofilin inactivation by overexpressing the upstream cofilin inhibitor LIM kinase slowed down wound repair by 33%, indicating that cofilin is necessary for rapid embryonic wound closure. We found that in controls, wounds transitioned from elliptical to circular in shape as tension increased around the wound. In contrast, cofilin inactivation caused wounds to remain elliptical, despite contractile force at the wound edge being similar to controls. In addition to severing filaments, cofilin can reduce actin fiber stiffness by up to a factor of four. Thus cofilin-induced actin severing, actin softening, or a combination of both could contribute to rapid wound closure. To distinguish between these possibilities, we developed an *in silico* molecular model of actomyosin cable contraction during wound healing. In the model, changing the degree of actin severing did not affect the rate of wound closure for a constant level of myosin motor activity. In contrast, a four-fold stiffening of the actin filaments slowed down wound healing by 64% and led to wounds that were significantly more elliptical. Together, our results suggest that

cofilin-mediated actin softening facilitates actomyosin cable contraction to drive rapid embryonic wound repair.

122 **Ribosomal proteins, Splicing and Cell Competition. Connecting the dots.** Eleni Tsakiri, Myrto Potiri, Kyriaki Kanakousaki, Martina Samiotaki, Panagiota Kafasla, Marianthi Kiparaki Biomedical Sciences Research Center «Alexander Fleming»

Cell competition phenomenon, where cells are eliminated via a non-autonomous mechanism in genetic mosaics but not in a homogeneous environment, was first documented in *Drosophila* between wild-type cells and cells with heterozygous dominant mutations in ribosomal protein (*Rp*) genes (known as *Minute* genes). In *Drosophila* 66 of the 79 *Rp* genes belong to the Minute class. During my tenure in Nicholas Baker's lab, we discovered that the transcription factor Xrp1 is responsible for almost all of the cellular responses of *Minute* cells, including reduced competitiveness, slower growth, reduced translation, and even for the developmental delay of the Minute flies. We identified the RpS12 protein (encoded by a non-*Minute* gene) mediating the Xrp1 activation. Interestingly, the RpS12-Xrp1 pathway is responsible for the competitive elimination of cells with segmental aneuploidies, since loss of chromosomes can lead to Rp gene haploinsufficiency. In addition, work from us and others revealed that Xrp1 and cell competition result from multiple other stresses (such as translation defects, ER stress), highlighting the significance of unraveling the mechanisms of Xrp1 activation. Ongoing work from my lab has revealed that RpS12 is involved in alternative splicing of the Xrp1 transcript both in wild-type and in *Minute* cells, producing the short isoform of Xrp1 protein. RpS12 overexpression in wild-type cells is sufficient to activate the Xrp1 pathway. We employ multiple approaches, including genome-wide alternative splicing analysis and proteomic analysis, along with classical genetics, to investigate the mechanism of splicing regulation of Xrp1 by RpS12 and the role of the two Xrp1 isoforms in $Rp^{+/-}$ responses. Our findings will contribute to the elucidation of the mechanisms of the Xrp1 pathway in cell competition, and additionally to the understanding of the underlying causes responsible for the Minute phenotype.

*Eleni Tsakiri (PhD) and Myrto Potiri (MSc) contributed equally in this work

123 **Cryo-EM structure of the retrotransposon Copia capsid hints at structural antagonism with dArc1 to regulate structural synaptic plasticity** Peter G M›Angale[1], Adrienne Lemieux[1], Yumeng Liu[2], Jasmine Graslie[1], Shuhao Wang[1], Brian Kelch[2], Travis Thomson[1] [1]Neurobiology, University of Massachusetts Chan Medical School, [2]Biochemistry & Molecular Biotechnology, University of Massachusetts Chan Medical School

Copia, a Drosophila retrotransposable element, has a physiological role at the Drosophila larval neuromuscular junction (NMJ) where it antagonizes the "master regulator" of synaptic plasticity, the activity regulated cytoskeleton-associated protein 1 (dArc1). Previous presynaptic downregulation of dArc1 resulted in a reduction in plasticity. Our current works shows that synaptic downregulation of Copia at the larval NMJ results in an increase in plasticity, marked by increased number of boutons and activity-dependent plasticity. Interestingly, we observe this phenotype when knocking down the precursor mRNA for the Copia capsid, as Copia is known to form viral-like particles much like dArc1. We determine there is an inverse relationship between Copia and dArc1, as the knockdown of dArc1 results in an increase in Copia mRNA and protein at the NMJ, while the inverse occurs with the knockdown of Copia. Interestingly, the knockdown of Copia in a dArc1 null background indicates that Copia is epistatic to dArc1 and the Copia phenotype is predominant to dArc1 as we observe an increase in plasticity in this genetic background. We observe that Copia acts antagonistically to dArc1 and together regulate structural synaptic plasticity at the larval NMJ. To elucidate the mechanism of Copia-dArc1 function, we employed single-particle cryo-EM methods, and resolved the structure of the Copia capsid to ~3.3-Å. From this structure we observe that Copia self-assembles into T=9 icosahedral capsid-like particles. We therefore formulate that the Copia-dArc1 antagonism could be at the monomer level, where the incorporation of either monomer to the nascent capsids results in the premature termination of the assembly, which we have named the "poison pill" model.

124 **Chromosome-level organization of the regulatory genome in the Drosophila nervous system**Xiao Li[1], Maria Gambetta[2], Michael Levine[1] [1]Princeton University, [2]University of Lausanne

Recent high-resolution 3D contact maps for the *Drosophila* brain have identified 58 meta-loops that hold together 28 pairs of distant topological associating domains (TADs) separated by distances of 1-20 Mb across individual chromosome arms. The meta-loops are formed by both boundary-boundary and tether-tether associations, although the *sns/hbs* TADs appear to be held together solely by tethering elements. *sns* and *hbs* are paralogs that encode similar cell adhesion molecules (CAM) containing Ig and Fn repeats, which are important for the formation of specific synapses in the fly brain. Larval brains obtained from mutants lacking the GAF POZ domain exhibit a specific reduction in sns/*hbs* interactions, resulting in diminished associations of an optic lobe enhancer in the *hbs* regulatory region with the *sns* promoter located ~6.2 Mb away. In addition, deletion of a putative enhancer within the *hbs* TAD led to decreased expression of *sns.* Many of the other meta-loops are also associated with CAMs, such as Dprs (defective proboscis extension response) and DIPs (Dpr-interacting protein). These

interactions are also controlled by sequence-specific tethering elements that work over vast distances and bypass intervening TADs. Understanding the specificity of these long-range interactions would open the door to chromosomal engineering efforts, whereby distant genes could be brought together in shared 3D hubs for coordinate regulation.

125 **Developmental remodeling repurposes larval neurons for sexual behaviors in adult *Drosophila*** Julia A Diamandi, Julia C Duckhorn, Kara E Miller, Mason Weinstock, Sofia Leone, Micaela R Murphy, Troy R Shirangi Villanova University

Most larval neurons in *Drosophila* are repurposed during metamorphosis for functions in adult life, but their contribution to the neural circuits for sexually dimorphic behaviors is unknown. Here, we identify two interneurons in the nerve cord of adult *Drosophila* females that control ovipositor extrusion, a courtship rejection behavior performed by mated females. We show that these two neurons are present in the nerve cord of larvae as mature, sexually monomorphic interneurons. During pupal development, they acquire the expression of the sexual differentiation gene, *doublesex*, undergo *doublesex*-dependent programmed cell death in males, and are remodeled in females for functions in female mating behavior. Our results demonstrate that the neural circuits for courtship in *Drosophila* are built in part using neurons that are sexually reprogrammed from former sex-shared activities in larval life.

126 **Toll-7 acts with Fra/DCC to promote commissural axon guidance across the midline** Sarah Gagnon[1], Yixin Zang[2], Greg J Bashaw[1] [1]Neuroscience, University of Pennsylvania, [2]Columbia

During embryonic development, commissural neurons send their axons across the central nervous system midline to connect to contralateral targets. In flies, the guidance receptor Frazzled (Fra), like its vertebrate ortholog Deleted in Colorectal Cancer (DCC), induces cytoskeletal rearrangements that facilitate growth toward the midline in response to its ligand Netrin. Fra also acts independently of Netrin to activate expression of *commissureless*, a negative regulator of the repulsive axon guidance receptor Roundabout. Fra therefore promotes midline crossing through Netrin-dependent attraction and Netrin-independent inhibition of repulsion. Elucidating the signaling mechanisms of Fra is essential to understanding how distinct receptor outputs are coordinated to achieve precise neural circuit wiring. We performed an affinity purification-mass spectrometry screen in fly embryonic neurons to identify novel Fra interactors and identified the toll-like receptor family member Toll-7. Like Fra, Toll-7 is expressed on commissural axons as they cross the midline. Previous work has shown that Toll-7 promotes motor and olfactory axon targeting, but its earlier developmental functions and potential role in midline circuit formation are unknown. Here we present evidence that Toll-7 acts with Fra to promote axon growth across the midline. Our work reveals that *toll-7* mutant embryos display axon guidance defects reminiscent of *fra* mutants, including defective projection across the midline and breaks in longitudinal axon tracks. Using a sensitized genetic background, we also find that like Fra, Toll-7 promotes midline crossing. Moreover, loss of a single copy of *toll-7* enhances the midline defects of mutants with attenuated Fra signaling, suggesting that Toll-7 promotes midline crossing through the Fra pathway. However, *fra, toll-7* double mutants show more severe midline crossing defects than *fra* single mutants, suggesting that Toll-7 plays additional, Fra-independent roles to promote midline crossing. In addition, biochemical results demonstrate that Fra and Toll-7 physically interact through their cytoplasmic domains. Future experiments will determine which Fra pathway Toll-7 acts through to promote midline crossing, and whether canonical Toll/NF-κB signaling underlies the Fra-independent function of Toll-7. Altogether, our work sheds light on uncharacterized mechanisms of Fra and Toll-7 signaling, providing insight into the assembly of neural circuits.

127 **LET-381/FoxF and UNC-30/Pitx2 control fate specification and maintenance of *C. elegans* mesodermal glia that regulate motor behavior.** Nikolaos Stefanakis[1], Jessica Jiang[1], Yupu Liang[1,2], Shai Shaham[1] [1]The Rockefeller University, [2]Alexion

Glia are cellular components of nearly all nervous systems and are anatomically positioned to affect every aspect of signal transduction and processing. While most glial cells are derived from neuroectodermal precursors, some, like microglia, are mesodermally derived. Mesodermal glia development is not well understood. *C. elegans* glia can broadly be divided into two classes: 46 sensory-neuron associated glia (sheath and socket cells) and ten synapse associated glia consisting of four CEPsh glia and six GLR glia cells, wrapping around the exterior and interior aspect of the *C. elegans* “brain” neuropil, respectively. Unlike sheath, socket and CEPsh glia that derive form a neuroepithelial lineage, GLR glia derive from a mesodermal-like lineage that mainly generates body wall muscle cells. To understand mesodermal glia development and functions we used FACS followed by RNAseq to identify the GLR transcriptome, and assessed how loss of GLR-enriched transcription factors affects GLR gene expression and morphology. Through temporally manipulated mutant analysis and CRISPR/Cas9 mutagenesis we exposed a regulatory network orchestrated by two transcription factors, LET-381/FoxF and UNC-30/Pitx, required for GLR glia development. LET-381/FoxF has an early role in specifying GLR glia fate and is also continuously required to maintain GLR gene expression by autoregulating its own expression. Using online motif analysis tools, we discovered putative *let-381* binding motifs in the promoters of several GLR expressed genes. Mutagenesis of such motifs disrupts endogenous GLR gene expression, suggesting that *let-381* directly controls GLR molecular identity. *unc-30/Pitx2* acts downstream of *let-381* to control GLR glia morphology and to repress gene expression of another mesodermal cell type in GLR glia. Together, *let-381* and *unc-*

30 are sufficient to induce GLR gene expression when ectopically expressed. We used the transcription factor mutants we identified to explore functions of GLR glia, whose anatomy and gene expression profile suggest involvement in motor behavior. Remarkably, GLR-defective animals display various motor behavior defects including increased reversal frequency and locomotory pausing. This study shows that like neurons, glia differentiation requires autoregulatory Terminal Selector genes that define and maintain glial fate. Transcriptome analysis raised the hypothesis that GLR glia may merge astrocytic and endothelial functions suggesting that GLR glia may represent a fate-merger combining characteristics of different vertebrate Blood Brain Barrier cells. Future studies deciphering roles of GLR glia in the function of the *C. elegans* nervous system may thus be crucial for a deeper understanding of the evolution of glia and brain barriers.

128 **A gene expression program induced by neuronal inactivity that regulates neuronal plasticity** Zhonghua Zhu[1], Jennifer Lennon[1], Seana Lymer[1], Justin Blau[2] [1]NYU Biology Department, [2]Biology, NYU Biology Department

Synaptic plasticity is essential for many processes including learning and memory, while defective plasticity is associated with neurological conditions such as autism spectrum disorder. We are using the dramatic daily structural plasticity of the Drosophila circadian pacemaker sLNv neurons to identify novel plasticity pathways.

Like many neurons, sLNvs use activity-regulated genes (ARGs) – also known as immediate early genes (IEGs) – that are rapidly activated via sLNv firing and increase the size of sLNv projections. We previously showed that rhythmic expression of the Rho1 GEF Pura drives the daily retraction of sLNv projections by rhythmically activating the Rho1 GTPase. Here we show that a Pura transcriptional reporter gene is repressed by neuronal activity and induced by hyperpolarization, which is the opposite of an ARG. Activation of Pura transcription by neuronal inactivity requires the transcription factor Toy, whose expression itself is also induced by hyperpolarization. Therefore, toy and Pura are inactivity-regulated genes (IRGs). We also show that toy transcription is induced by decreasing intracellular calcium – again opposite to ARGs. Thus intracellular calcium levels seem to represent two cellular states that act as a binary switch to control distinct gene expression programs: high calcium induces ARGs while low calcium induces IRG expression. Toy is an ortholog of mammalian Pax6, which is expressed in many adult neurons. We propose that inactivity-regulated genes are a general property of plastic neurons.

129 **Balancing act: exploring pH dynamics in stem cell fate regulation** Bernice Lin[1,2], Isabella Maag[1], Ashley Bielawski[1], Beverly Piggott[1,2,3] [1]Division of Biological Sciences, University of Montana, [2]Neuroscience, University of Montana, [3]Center for Biomolecular Structure and Dynamics, University of Montana

It has traditionally been held that cells strictly maintain their pH within a narrow physiological range, with deviations occurring mainly in disease states like cancer (leading to a more basic pH) or neurodegeneration (resulting in a more acidic pH). However, an increasing body of evidence suggests that different cell types, across species, do not merely regulate their pH to prevent pathology but actively fine-tune their pH levels to govern molecular interactions and cellular behaviors. Sodium (Na^+) proton (H^+) exchangers (Nhes) play a pivotal role in intracellular pH (pHi) regulation by facilitating the efflux of H^+ ions in exchange for the influx Na^+ ions. The influx of Na^+ ions has been observed to influence mitotic swelling due to hydrostatic interactions, thereby elevating pHi. While it has long been supported that a relatively basic pHi supports cell proliferation, the extent to which cells inherently control pH to optimize molecular mechanisms of asymmetric division remains elusive. *Drosophila* neuroblasts (NB), the neural stem cells of the *Drosophila* brain, serves as a powerful model to study neural stem cells *in vivo* thanks to their highly conserved mechanisms and precise genetic tools. While there are nine NHE (NHE1-9) proteins in humans, there are only three in *Drosophila* (*dnhe1-3*), providing a straightforward model to study the role of Nhes in neurogenesis. Our preliminary data has identified critical roles for Nhe proteins in brain development. We find that loss or knockdown of Nhes reduces proliferation. Using a genetically encoded pH sensor, we find that NB are more basic than their differentiated progeny. Our data suggests that Nhes may also regulate cellular division machinery, as Nhe knockdown manifests in defects that result in smaller brain sizes. Our hypothesis posits that Nhe proteins regulate stem cell fate and proliferation by maintaining a basic pHi that supports neuroblast physiology and behavior. Given that mutations in human NHEs are associated with various neurodevelopmental disorders like Christianson syndrome, microcephaly, and cognitive impairment, these findings may offer valuable insights for future therapeutic interventions.

130 **Ancient enlargement of complex neuronal genes** Matthew J McCoy[1], Andrew Z Fire[2] [1]Pathology, Stanford University School of Medicine, [2]Pathology and Genetics, Stanford University School of Medicine

The evolutionary origins of nervous systems and their complex wiring are key questions in biology. This study investigates a distinct class of extremely large, multi-isoform genes enriched for expression in neurons at synapses. These genes, often spanning hundreds of thousands to millions of base pairs in the genome, are frequently mutated or misregulated in neurological disorders. While many of these genes have been studied individually, it remains unknown whether there are any overarching principles governing their parallel evolution. To address this, we conducted a comparative analysis of orthologous

gene sizes across diverse eukaryotes. Our findings reveal a unique class of large genes predating animal diversification and in many cases the evolution of neurons. We traced this class of ancient large genes through evolution and found orthologs of the large synaptic genes implicated in driving the immense complexity of metazoan nervous systems, including in humans and cephalopods. Notably, these genes exhibit low dN/dS scores, indicating strong purifying selection, yet have simultaneously undergone enlargement and gained numerous isoforms in animals. This research provides a new lens through which to view the evolution of this distinctive gene class, and highlights how intrinsic genomic properties, like gene length, can provide flexibility in molecular evolution and allow groups of genes and their host organisms to evolve toward complexity.

131 **Elucidating shared and dimorphic circuitry regulating aggression in *Drosophila*** Catherine E Schretter[1], Hui Chiu[2,3], Mei Shao[1], Alice A Robie[1], Kristin M Branson[1], David J Anderson[2], Gerald M Rubin[1] [1]Janelia Research Campus of HHMI, [2]California Institute of Technology, [3]Yale

The regulation of aggression has important implications for survival as its engagement risks injury and alters group dynamics. Both male and female fruit flies, *Drosophila melanogaster*, display aggression, yet there are sex-specific differences in component behaviors and environmental contributions. Previous work by our lab and others implicated two neuronal populations in aggression, CAP and aIPg, which modulate approach behavior in both sexes and female aggressive behavior, respectively (Chiu et al., 2021; Schretter et al., 2020). However, it has previously been difficult to elucidate the connections between such shared and sexually dimorphic neuronal mechanisms due to the lack of a complete circuit diagram.

In the present study, we used the female fly connectome to map the connections between these cell types, elucidating the circuit components and environmental variables that underly monomorphic and dimorphic components of aggression. Further, we uncovered a novel glutamatergic cell type that appears to downregulate aggression in both sexes. In addition to furthering our understanding of aggressive behaviors, this work has important implications for uncovering how the brain flexibly regulates social behaviors in both sexes.

132 **Experience-dependent control of sex-specific cadherin expression defines sexually dimorphic neurite adjacency and synaptic connectivity** Chien-Po Liao[1], Maryam Majeed[2], Oliver Hobert[3,4] [1]Biological Sciences, Columbia University, [2]Allen Institute for Brain Science, [3]Biological Science, Columbia University, [4]Howard Hughes Medical Institute

Juvenile experiences can shape sexually dimorphic synaptic connectivity in the nervous system of the nematode *C. elegans*. We reveal here the molecular mechanisms that establish the juvenile starvation-dependent and sex-specific innervation of the AVA interneuron by the PHB sensory neuron. We show that food-dependent serotonin signaling in the PHB sensory neuron of juvenile animals establishes proper expression of the Zn finger transcription factor LIN-29A via G-protein coupled 5HT receptor signaling and the CREB transcription factor, specifically in male animals. Through the regulation of an intermediary, Doublesex-like transcription factors, DMD-4, male-specific LIN-29A represses the expression of an atypical cadherin protein FMI-1/Flamingo in PHB. In hermaphrodites, FMI-1 promotes the physical adjacency of the PHB and AVA neurites thereby enabling *en passant* synaptic innervation. The experience- and serotonin-dependent promotion of LIN-29a expression in male PHB results in FMI-1 downregulation, resulting in decreased PHB/AVA adjacency and therefore limiting PHB>AVA connectivity in males. Our findings reveal the dynamic control of neurite adjacency as a mechanism for controlling dimorphic synaptic connectivity.

133 **Regulation of Sex Differences in Innate Immunity by Sex-Determining Gene *transformer* in *Drosophila*** MD Mursalin Khan, Rita Graze Biological Sciences, Auburn University

Sex dimorphism is a common fundamental characteristic of sexually reproducing organisms, exerting influence over nearly all aspects of life history. However, the regulation of genes underlying sex dimorphism is underexplored for many complex traits, including immunity. To investigate the regulatory basis of sex dimorphism in immunity in Drosophila, we focused on a key switch gene of the sex determination hierarchy, *transformer (tra)*. Animals with wild-type sex determination, XX and XY, were compared to *tra*-mutant animals lacking functional Tra and *tra*-overexpression animals expressing Tra ubiquitously. Survival, bacterial load, and transcriptomic profiles were assessed in controls and upon infection with *Providencia rettgeri* Dmel, a pathogenic strain collected from *Drosophila melanogaster*. To the best of our knowledge, this is the first genomic analysis delineating the nature and extent of the role of *tra* in regulating sex dimorphism of the immune response in insects. We find that *tra* likely regulates observed sex differences in survival as well as sex differences in the expression of core innate immune response pathways. Our study identifies a significant regulatory role of *tra,* or its downstream targets, in the expression of genes involved in Toll signaling with significant enrichment, including signal detection, transduction, antimicrobial peptides, and negative regulators of the pathway. We also find key genes in the IMD pathway show patterns consistent with regulation by or downstream of *tra*, but genes in this pathway are not significantly enriched amongst the putative targets identified in this analysis. In addition, the overexpression of Tra^F in XY animals results in a surprising outcome, much higher survival relative to both XX and XY controls, and an altered pattern of bacterial load over time. This suggests that increased dosage or the altered expression pattern of Tra^F in *tra*-overexpression animals drives increased efficiency of the immune response. Overall,

our results point to a complex role of the sex determination pathway in modulating sex dimorphism of the immune response and suggest a need for further exploration of the direct regulators and individual targets, as well as the dynamics of expression during infection.

134 **Functional genomics of sexual dimorphism : Doublesex mediates sex-specific control of ultraviolet iridescence in sulphur butterflies** Ling Sheng Loh[1], Martina Tsimba[2], Joseph J Hanly[2,3], Arnaud Martin[2] [1]Biology, George Washington University, [2]George Washington University, [3]Duke University

What is the genetic regulatory logic behind the elaboration of sexually dimorphic traits during evolution? To answer this question, we are deciphering the regulatory factors behind the development of ultraviolet iridescence in sulphur butterflies, a color signal involved in male courtship displays. Given that this trait is absent or found in rudimentary states in females, it is likely maladaptive in this sex, presumably due to the added conspicuity to bird predators with UV vision. Based on phylogenetic analyses, it is likely that the evolution of UV-iridescence was accompanied by regulatory mechanisms to repress the UV trait in females. In the orange sulphur butterfly Colias eurytheme, UV color scales are repressed by the Bric-a-brac (Bab) transcription factor, but it remains unclear how the expression of this gene became sexually dimorphic. Here we sought to elucidate this question using single-nuclei transcriptomics, ChIP-seq, fluorescent mRNA/protein stainings, and CRISPR/Cas9 induced gene knock-outs. Single-nuclei transcriptomes in four time points during pupal wing development revealed upstream components in UV cell fate determination. We found that Doublesex (Dsx) and bab are expressed in a mutually exclusive manner in wing scale precursor cells, implying that Dsx repressed Bab in males. ChIP-seq profiling of these two modulators of sexual differentiation further refines the potential target genes involved in UV-scale differentiation, including the identification of negative feedback loops reinforcing somatic sex identity. Functional knockouts confirmed the roles of Dsx and bab in the binary decision of recruiting or repressing the UV scale-making network, bringing in the interaction of sex-determinating factor dsx and a sex-linked gene bab in regulating an ancestral gene network underlying a sexually dimorphic trait.

135 **The TGF-β hormone *amh* is a convergently evolved sex determination gene in threespine stickleback (*Gasterosteus aculeatus*)** Matthew Treaster, Michael White Department of Genetics, University of Georgia

Master sex determination genes have evolved independently in many taxa. Across vertebrates, a wide variety of genes are used to initiate the critical developmental process of sex determination. However, the gene *anti-mullerian hormone* (*amh*) has convergently evolved as a sex determination gene in multiple species of teleosts. Ancestrally, *amh* is dispensable for male determination, thus how *amh* initiates testis development in some species and how it repeatedly evolves this novel function is not known. In threespine stickleback (*Gasterosteus aculeatus*), *amh* was duplicated onto the Y chromosome (*amhy*) approximately 20 million years ago. To determine whether the Y-linked *amhy* is the master sex determination gene in this species, we have targeted two different exons with CRISPR/Cas9 to knock out *amhy*. Histology of XY *amhy*-KO gonads shows complete male to female sex reversal. XY *amhy*-KO fish produce fully developed ovaries with mature oocytes rather than testes, demonstrating that *amhy* is necessary for male sex determination. To test whether *amhy* is sufficient for male sex determination, we have introduced a CMV-driven *amhy* transgene into XX fish and will analyze these XX *tg(CMV:amhy)* fish for female to male sex reversal. Preliminary characterization of the transcriptome across development shows that *amhy* is expressed more highly than its autosomal paralog, *amh08*, in larval stickleback while *amh08* is expressed more highly in adult testes. This expression divergence highlights clear regulatory evolution that has occurred during neofunctionalization of *amhy* as a sex determination gene. To identify the core regulatory elements responsible for this novel function, we have created a transgene consisting of *amhy* and its surrounding non-coding sequence from the Y chromosome. Our findings establish threespine stickleback as a powerful model to investigate how *amh* regulates gonadal development and how this gene repeatedly evolves novel function as a master sex determination gene.

136 **The RNA-binding protein, Rbpms2, regulates mTOR signaling via the GATOR2 complex protein, Mios, to promote oogenesis and female fate in zebrafish** Miranda L Wilson[1], Shannon Romano[1], Nitya Khatri[1], Devora Aharon[1], Yulong Liu[2], Odelya Kaufman[3], Bruce Draper[2], Florence Marlow[1,3] [1]Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, [2]University of California, [3]Albert Einstein College of Medicine

Differences of sexual development (DSDs) occur in 1:5000 live births and encompass various congenital conditions with diverse phenotypes resulting from genetic, developmental, or hormonal anomalies that arise during embryogenesis and persist throughout sexual development into adulthood. RNA binding proteins (RNAbp) control post-transcriptional gene expression and have been implicated in sex determination and DSDs. We previously identified the vertebrate specific RNAbp of multiple splice forms 2 (Rbpms2) as a positive regulator of female sex determination in zebrafish. To determine how Rbpms2 promotes oocyte development, we isolated Rbpms2-bound RNAs in adult ovaries (*rboRNAs*). We find that these *rboRNAs* are associated with testis and ovary fates, suggesting Rbpms2 functions to both repress testis and promote ovarian developmental pathways. Further, our data indicate Rbpms2 regulates *rboRNAs* via translational control. We evaluated potential ovary

promoting *rboRNAs* and their associated pathways, particularly ribosome biogenesis-related targets as *rbpms2*$^{-/-}$ oocytes have nucleoli deficits. Specifically, we investigated the Gator2 component Mios (missing oocyte), which activates the ribosome biogenesis-associated mTorc1 pathway. We show that *mios* contains Rbpms binding sites in its 3' UTR and that Mios is absent in *rbpms2*$^{-/-}$, placing Mios downstream of Rbpms2. Further, *mios*$^{-/-}$ have nucleoli deficits and develop exclusively as fertile males. To evaluate mTorc1 signaling in ovary determination, we genetically reduced negative regulators, *SPO11 initiator of meiotic double stranded breaks* (*spo11*) and *TSC complex subunit 2* (*tsc2*), in *mios*$^{-/-}$. We show that loss of *spo11*, unlike in *Drosophila* (*mei-W68*), failed to restore oogenesis in *mios*$^{-/-}$. Similarly, reducing Tsc2 or constitutive activation of its downstream target Rheb (Ras homolog, mTor binding) was insufficient to suppress oocyte loss. Conversely, germline expression of constitutively active *mTOR* restored oogenesis in *mios*$^{-/-}$. Cumulatively, our results indicate early gonocytes are in a poised bipotential state where an RNAbp-mediated binary fate switch dynamically regulates testis and ovary-specific factors and activates a Gator2-mediated oogenesis checkpoint, integrating sexual differentiation and nutrient status during zebrafish sex determination. Further, our findings have broader implications for understanding the genetic mechanisms regulating sex determination, differentiation, and DSD etiology in humans.

137 **Combining transcriptomic and epigenetic data to understand the sources of sex-specific ageing in bats** Jack Rayner, Danielle M. Adams, Gerald Wilkinson Department of Biology, University of Maryland, College Park

Lifespan differs dramatically across taxa, but extensive variation in size, life history, and large phylogenetic distances complicate interpretation of these differences. Lifespan is also frequently found to differ drastically between sexes of the same species, a nuance that is often missing from phylogenetic comparisons. Given that sexes largely share a genome, exaggerated differences in sex-specific survival offer a promising avenue for illuminating non-genetic factors associated with ageing and longevity. Bats are a model taxon for ageing research, typically living considerably longer than other mammals of a comparable size. However, as in many fields of biology, sex differences in longevity of bats and other species are often overlooked. These sex differences are sometimes striking: for example, we find that females of the greater spear-nosed bat live more than twice as long as males of the same species. To address this, we have collected gene expression and methylation data from males and females of multiple bat species, across a representative range of ages, to compare transcriptomic and epigenetic signatures of ageing. By incorporating intra- and interspecific sources of variation, we can begin to disentangle forces of sexual and natural selection underlying patterns of ageing and longevity in this exceptionally long-lived lineage. For instance, in species exhibiting striking sex-differences in longevity, accelerated patterns of ageing and biological dysfunction in the shorter-lived sex might illustrate the effects of divergent life history strategies shaped by sexual selection. Our findings will inform understanding of the evolutionary forces shaping age-related variation and lifespan, with particular attention to sex differences, and contribute to knowledge of signatures and potential of ageing more broadly.

138 **Ancient structural variation controls heterodichogamy across Juglandaceae** Jeffrey Groh[1], Kristian Stevens[2], Pat J. Brown[3], Charles H. Langley[1], Graham Coop[1] [1]Evolution and Ecology, University of California, Davis, [2]Computer Science, Evolution and Ecology, University of California, Davis, [3]Plant Sciences, University of California, Davis

The maintenance of stable mating type polymorphisms is a classic example of negative frequency dependent sexual selection, the principle underlying nearly ubiquitous 50/50 sex ratios in species with separate sexes. One lesser known but intriguing example of sex ratio selection resulting in a balanced mating polymorphism in some angiosperms is heterodichogamy - polymorphism for opposing directions of sequential hermaphroditism. While rare and poorly understood, this mating system is characteristic of Juglandaceae, the family that includes important nut and timber crops, the walnuts (*Juglans*), pecans and hickories (*Carya*). We show that the genetic locus controlling alternate flowering morphs (H-locus) in *Juglans* is an ancient balanced polymorphism that arose in the common ancestor of the genus, ~60 Mya. The *Juglans* H-locus maps to a ca. 10kb structural variant adjacent to a flowering time gene, a homolog of which regulates flowering time in Arabidopsis. An insertion in the dominant (protogynous) haplotype contains 8-12 complex tandem duplicates of the 3' UTR of the gene, whereas the recessive haplotype contains a derived CACTA-like DNA transposon. A phylogeny of repeats in the protogynous haplotype reveals both deep conservation of ancestral repeats as well as lineage-specific repeat turnover. We examine the functional relevance of these repeats for gene expression of the candidate flowering gene. We find that the H-locus is not shared across genera within Juglandaceae, and that a separate, non-syntenic structural variant controls flowering morphs in *Carya*. Finally, we discuss potential mechanisms underlying the lability of the genetic basis for heterodichogamy.

139 **The Interplay of peroxisome and mitochondrial dynamics during aging in Drosophila melanogaster** Ankur Kumar[1], Hua Bai[2] [1]Genetics Development and Cell Biology, Iowa State University, [2]Iowa State University

Healthy mitochondria play an essential role in maintaining cellular homeostasis. It is known that mitochondrial structure and function are impaired during aging, likely due to dysregulated mitochondrial fission and fusion processes. Through TEM and confocal imaging analysis, we find that mitochondrial size increases in aged oenocytes, the hepatocyte-like cells in Drosophila. Interestingly, we find that mitochondria from aged oenocytes do not exhibit paraquat-induced mitochondrial fission in contrast

to young flies. Additionally, aged flies have decreased numbers of mitochondria but increased size compared to young flies. Our preliminary studies suggest impairment in peroxisomal function in adult oenocytes results in mitochondrial morphology alterations similar to the old flies. However, peroxisomal impairment in indirect flight muscle did not show mitochondrial morphology alteration.Further imaging analysis showed that muscles have fewer peroxisomes than the oenocytes, which again supports the involvement of peroxisomes in regulating mitochondrial morphology. Therefore, we screened peroxisomal genes; among those, only Gnpat showed similar mitochondrial morphology. Further, we find that the knockdown of plasmalogen biosynthesis enzymes (e.g., Gnpat and Kua/PEDS1 Glyceronephosphate O-acyltransferase) blocks paraquat-induced mitochondrial fission and the recruitment of fission factor Drp1 to mitochondria, which was also confirmed by utilizing our newly developed live cell imaging technique. We discovered the decrease of mitochondrial plasmalogen C18:0/C18:1-PE aged flies using Mass spectrometry. To discover the involvement of plasmalogen in mitochondrial fission, we performed the lipid pull-down assay using synthetic biotinylated C18:0 plasmalogen PE (pPE) and identified interacting mitochondrial fission proteins such as Drp1 and Mff. To further validate the interacting partner, we pulled down the crude mitochondrial extract, which showed that the Drp1 and Mff interact with biotinylated pPE. Together, our findings suggest that the peroxisomal plasmalogen synthesis pathway plays an important role in maintaining normal mitochondrial health during animal aging.

140 **Defective phagocytosis leads to neurodegeneration through systemic increased innate immune signaling in *Drosophila*.** Guangmei Liu, Johnny Elguero, Shruthi Bandyadka, Zhenhao Yan, Cheng Yang Shi, Iqra Amin, Kim McCall Boston University

In nervous system development, disease, and injury, neurons undergo programmed cell death, leaving behind cell corpses that are removed by phagocytic glia. Altered glial phagocytosis has been implicated in several neurological diseases including Alzheimer's disease, Parkinson's disease, and traumatic brain injury. To untangle the links between glial phagocytosis and neurodegeneration, we investigated *Drosophila* mutants lacking the phagocytic receptor, Draper. Expressed on the membrane of phagocytes, Draper plays a crucial role in recognizing and engulfing dead cells and debris. Loss of Draper in glia leads to persistent neuronal cell corpses and age-dependent neurodegeneration. The presence of uncleared cell corpses and debris can act as activators to trigger increased immune signaling. We wondered whether neurodegeneration observed in *draper* mutants results from heightened immune activity. A major immune response in *Drosophila* is the activation of two NFκB signaling pathways that produce antimicrobial peptides, primarily in the fat body. In alignment with our hypothesis, we found that the antimicrobial peptide Attacin A is highly upregulated in the fat body of aged *draper* mutants and that inhibition of the Immune deficiency (Imd) pathway in the glia and fat body of *draper* mutants led to reduced neurodegeneration, indicating that immune activation promotes neurodegeneration in *draper* mutants. Immune activation in the central nervous system is characterized by the presence of activated, pro-inflammatory glia as well as infiltrating peripheral immune cells. Thus, we wondered if peripheral immune cells, hemocytes, react to glial phagocytic defects and invade into brain as flies age. We utilized hemocyte reporter line in *draper* mutants and found that hemocytes cluster at the pars intercerebralis in the central brain. To further dissect the role of immune signaling in peripheral (e.g., fat body and hemocytes) and local (e.g., glia) tissues in promoting age-dependent neurodegeneration in *draper* mutants, we performed snRNA-seq in fly heads to delineate differentially transcribed mRNAs in these tissues. snRNA-seq data will be presented in the conference. Taken together, these findings indicate that phagocytic defects lead to neurodegeneration via increased systemic and local immune signaling.

141 **Probing the mechanism of ROS-induced glial lipid droplet formation and implications for Alzheimer's disease** Matthew Moulton[1], Scott Barish[1], Isha Ralhan[2], Jinlan Chang[2], Lindsey Goodman[1], Jake Harland[1], Paul Marcogliese[3], Maria Ioannou[2], Hugo Bellen[1] [1]Molecular & Human Genetics, Baylor College of Medicine, [2]Physiology, University of Alberta, [3]Biochemistry and Medical Genetics, University of Manitoba

With an ever-growing list of Alzheimer's disease (AD) genetic risk factors, it is increasingly important to delineate the contribution of risk alleles on disease mechanisms. The apolipoprotein, APOE, is the highest known genetic risk factor for AD, implicating lipid dysregulation in AD pathogenesis. We have developed a model of reactive oxygen species (ROS)-induced glial lipid droplet (LD) formation and neurodegeneration in the fly. We have shown that elevated ROS in neurons triggers the production of lipids that become peroxidated by ROS. Peroxidated lipids are shuttled out of neurons and taken up by glia where they accumulate in LDs. Glial LD formation requires expression of an ortholog of APOE, *Glial Lazarillo* (*GLaz*). Targeted replacement of *GLaz* with expression of human variants of APOE demonstrates that the AD-associated allele, APOE4, reduces glial LD formation and promotes neuronal demise but APOE2 and APOE3 both facilitate LD formation and protect against neurodegeneration. We have identified additional genes required for glial LD formation that overlap with AD risk loci identified in genome wide association studies including *eato* (*ABCA1*), *ldd* (*ABCA7*), *LRP1* (*LRP1*), *VPS26* (VPS26A/B), *VPS35* (VPS35), *AP-2α* (AP2A2), *lap* (*PICALM*), and *cindr* (*CD2AP*). We now seek to understand the effects of variation in these AD risk genes on lipid transport between neurons and glia using novel humanized fly models.

Lipid dysregulation may also affect Aβ42-induced neurotoxicity. APOE4 carriers have higher rates of amyloid deposition and

lower rates of amyloid clearance than non-carriers, suggesting an interplay between lipid transport and Aβ42 clearance. Aβ42 is a lipophillic molecule that binds to APOE and an APOE receptor, LRP1. Thus, we hypothesize that LD formation in glia may contribute to the clearance of Aβ42 by promoting its cellular uptake and degradation. In the presence of ROS, however, we demonstrate that the neurotoxic effects of Aβ42 are enhanced in both fly and mouse models suggesting that efforts to mitigate Aβ-inducted neurotoxicity should be coupled with ROS mitigation strategies. We further demonstrate that ROS-mediated neurodegeneration can be abrogated by the use of a blood-brain-barrier penetrating antioxidant, N-acetylcysteine amide. Altogether, our data implicate a link between lipid uptake, ROS, Aβ production, and neurodegeneration and suggest that ROS mitigation could be an important therapeutic strategy for AD.

142 **Dissecting cutaneous wound healing in zebrafish** Leah Greenspan[1], Keith Ameyaw[2], Daniel Castranova[1], Van Pham[1], Gennady Margolin[3], Caleb Mertus[3], Brant Weinstein[1] [1]NICHD, National Institutes of Health, [2]Albert Einstein College of Medicine, [3]National Institutes of Health

Two percent of the US population is plagued by open chronic wounds, with a delay in vascular reperfusion being a major contributor to wound closure defects. This delay often occurs in aged or diabetic adults resulting in impaired healing, yet how and why this delay occurs remains unclear. Mammalian models have provided insight into the cell types and signals driving different phases of wound healing, but these models do not permit high-resolution imaging of the healing process in living animals, making it difficult to study transient events and cell-cell interactions. Zebrafish are ideal for visualization and experimental dissection of cutaneous wound healing in the context of an intact, living animal, with numerous transgenic lines available that mark relevant cell populations and powerful methods for high-resolution imaging of these lines. We have established a reproducible cutaneous wound model in zebrafish using a rotary tool combined with cellular-level long-term confocal imaging of wounds in living adult fish. We find that skin re-epithelialization and neutrophil recruitment initiates within hours after injury and peaks at one day, while macrophage activity and vessel regrowth increase between 1-4 days post injury, and vessel re-patterning takes many additional months. We are currently using our new model to study how wound healing is altered in aging and diabetic fish. We have also successfully established a method for *in vivo* profiling of endothelial cells using TRAP-RNAseq of “AngioTag” transgenic fish, demonstrating that this method can be used to uncover tissue- or organ-specific endothelial gene expression signatures. We are now using this technique to study the endothelial gene expression changes that occur during wound healing and how these are altered in the elderly and diabetics. Together, these studies will uncover the cellular and molecular mechanisms that restore vascular networks after cutaneous injury, providing potential new targets for therapeutic approaches.

143 **Tissue-specific temporal responses to aging in *Drosophila melanogaster* transcriptome** Maryam Nasiri Aghdam[1,2], Vijay Shankar[3], Maria E Adonay[3], Desireé Unselt[4], Robert Anholt[3], Trudy Frances Charlene Mackay[3] [1]Clemson University, [2]Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, [3]Clemson University Center for Human Genetics, Clemson University, [4]Q² Solutions

The aging process encompasses intricate molecular dynamics across various tissues, exerting a significant influence on lifespan. *Drosophila melanogaster*, characterized by its swift generation cycles and controlled laboratory conditions, provides an optimal model for investigating these intricacies. Nonetheless, our understanding of tissue-specific molecular alterations during aging in this species remains limited. Employing longitudinal transcriptomic analysis on an advanced intercross population (AIP) of *D. melanogaster* across 11 time points and in diverse tissues (head, body, and reproductive), we discerned temporal trends in gene expression profiles. Time-dependent shifts from day 5 revealed a prevailing downward trend in male tissues and female body tissue, while female head and reproductive tissues exhibited an upward trend. A decline in key pathways, such as the tricarboxylic acid (TCA) cycle and amino acid biosynthesis, is consistently observed across both male and female tissues. An upregulation in xenobiotic metabolism through cytochrome P450, as well as galactose and glutathione metabolism, is shared across the sexes. In contrast, specific pathway modifications exhibit tissue-specific patterns. For instance, the sphingolipid metabolism pathway demonstrates an exclusive elevation in the female head tissue, while the DNA replication and repair pathways manifest an increase solely in the male head tissue. Furthermore, our analysis revealed differential regulation of pathway expression, with some tissues displaying an increase while others exhibit a decrease in overall pathway expression. Among the genes exhibiting the most substantial age-related variation, we identified alterations in the expression of *trbd*, *Mic26-27*, *SdhD*, *SERCA*, and *Ide*, which have previously been associated with lifespan. Additionally, we uncovered new age-related candidates with tissue-specific expression over time, including *Arc1*, *UQCR-C1*, *Apt1*, and *IBIN*. In summary, the aging process elicits distinct responses within various tissues. Notably, essential pathways like the TCA cycle and redox signaling continue to play crucial roles in determining lifespan in Drosophila. Finally, the emergence of novel age-related genes offers valuable insights into unexplored complexities associated with the aging process.

144 **Addressing Mendelian neurodegeneration: genome engineering of mouse models for X-linked dystonia-parkinsonism** Yu Jeremy Zhao[1], Weimin Zhang[1], Priya Prakash[2], Heather Appleby[1], Yinan Zhu[1], Ran Brosh[1], Shane Liddelow[2], Jef

D. Boeke[1] [1]Institute for Systems Genetics, NYU Langone Health, [2]NYU Langone Health

X-linked Dystonia-Parkinsonism (XDP) is a severe hereditary form of Parkinsonism endemic to the Philippines, caused by an SVA (SINE-VNTR-Alu) retroelement insertion in the *TAF1* gene that disrupts its normal splicing and expression. The specificity of XDP to human gene structure and the complexity of repetitive retroelement sequences present significant obstacles in creating accurate mouse models through direct genome editing.

Leveraging our expertise in large-scale DNA assembly, we aim to address this challenge with a genome writing approach. We engineered mouse embryonic stem cells (mESC) to include an XDP patient-derived *TAF1* gene downstream of the mouse *Taf1*. Upon Cre-mediated recombination, this locus converts by replacing exons 25-38 with a hybrid region that combines mouse protein-coding sequences and human gene structure. Our *in vitro* analyses confirm that the "humanized" *Taf1* gene undergoes proper splicing and preserved multiple known mouse and human splice isoforms. Utilizing these modified mESCs, we generated pre-converted TAF1/XDP mouse models and subsequently crossed them with Cre drivers to induce tissue-specific conversions. *In vivo* characterization revealed that neuronal-specific expression of the hybrid *Taf1* with the SVA insertion dramatically reduced brain size, enlarged lateral ventricles, and caused significant striatal and hippocampal pathology. Moreover, XDP male mice displayed irregular gait and reduced motor performance, similar to human XDP patients' symptoms.

We will introduce additional genetic variants, including age-related hexanucleotide repeats within the SVA element, to explore their inverse relationship with disease onset. Furthermore, this project represents our capability to design, construct, and deliver large DNA constructs (>100 kb) for genome writing in mammalian systems. Ultimately, our work sets the stage for addressing complex biological questions and genetic variations, establishing a foundation for collaborative exploration.

145 **Uncovering shared and neuron-specific targets across ALS and FTD relevant circuits in Drosophila models of TDP-43 proteinopathy** R. Keating Godfrey[1,2], Eric Alsop[3], Reed T Bjork[4], Lauren M Gittings[5], Rita Sattler[5], Kendall Van Keuren-Jensen[3], Daniela C Zarnescu[6,7] [1]University of Florida, [2]Department of Biological Sciences, Florida International University, [3]Translational Genomics Research Institute, [4]Department of Neuroscience, University of Arizona, [5]Dept. of Translational Neuroscience, Barrow Neurological Institute, [6]Cellular and Molecular Physiology, Penn State College of Medicine, [7]Department of Molecular and Cellular Biology, University of Arizona

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) comprise a spectrum of neurodegenerative diseases linked to TDP-43 proteinopathy, defined as the accumulation of cytoplasmic TDP-43 inclusions accompanied by loss of nuclear TDP-43. Proteinopathy ultimately causes RNA processing defects including dysregulation of splicing, mRNA transport and translation. Complementing our previous work in motor neurons, here we report a novel model of TDP-43 proteinopathy based on overexpression of TDP-43 in a subset of *Drosophila* Kenyon cells of the mushroom body (MB). This model recapitulates several aspects of dementia-relevant pathological features including age-dependent neuronal loss, nuclear depletion and cytoplasmic accumulation of TDP-43, and behavioral deficits in working memory and sleep that occur prior to axonal degeneration. Using RNA immunoprecipitations, we identify several candidate mRNA targets of TDP-43 in MBs, some of which are unique to the MB circuit and others that are shared with motor neurons that we have previously reported. Among the latter is the glypican Dally-like-protein (Dlp), which exhibits significant TDP-43 associated reduction in expression during aging. Bioinformatic analyses of candidate mRNA targets associated with TDP-43 in MBs highlight several signaling pathways including Hippo and wingless among others. Using genetic interaction approaches we show that overexpression of Dlp in MBs mitigates TDP-43 dependent working memory deficits, consistent with Dlp acting as a mediator of TDP-43 toxicity. Substantiating our findings in the fly model, we find that the expression of *GPC6* mRNA, a human ortholog of *dlp,* is specifically altered in neurons exhibiting the molecular signature of TDP-43 pathology in FTD patient brains. Furthermore, we identify several MB specific candidate targets of TDP-43 that are also altered in FTD patient brains and encode proteins associated with transcription, synaptic activity and proteasome mediated degradation. These findings suggest that circuit-specific *Drosophila* models provide a platform for uncovering shared or disease-specific molecular mechanisms and vulnerabilities across the spectrum of TDP-43 proteinopathies.

146 **Profiling Transcriptomics and Chromatin Accessibility Reveals Molecular Changes Associated with Protective Effects Induced by Priming** Hsin-Yun Chang, Siu Sylvia Lee Cornell University

Hormetic stress, where exposing the body to mild doses of stress, such as hot saunas and intermittent fasting, is thought to improve its resilience during later challenging times and have been popularized as health promoting regimens. While this phenomenon is well-documented across species, much remains to be learnt about the underlying molecular mechanisms, particularly regarding how priming can improve stress resilience and confer physiological benefits over a range of timescales. In this study, we developed a robust regimen where *C. elegans* were "primed" by transient exposure to mild heat stress, followed by recovery, and subsequent lethal heat shock challenge. We observed that the primed worms show consistently heightened

resistance to a later heat shock challenge, including better survival and motility, compared to naive worms. To investigate the possible gene regulatory mechanisms of this priming-induced protective effects, we performed ATAC-seq and RNA-seq at different timepoints, including immediately after priming, after recovery, and upon heat shock challenge. We hypothesized that changes in both chromatin accessibility and RNA expression would provide insights into possible molecular mechanisms of the protective effects. We found that mild heat stress priming and heat shock challenge both induce substantial changes in chromatin accessibility and RNA expression. Interestingly, the priming-induced changes are largely distinct from those induced by heat shock, suggesting that different levels of heat stress induce distinct molecular changes. Excitingly, while most priming-induced changes are restored after recovery, primed and naïve worms exhibited significant differential chromatin accessibility and RNA expression upon heat shock challenge. These differences likely point to candidate genes that contribute to the priming-induced beneficial effects. We have initially focused on the genes that showed correlated changes in chromatin accessibility and RNA expression in the primed worms, which include annotated transcription and chromatin factors, and lipid metabolism regulators. We are continuing with data comparison and functional testing of the identified candidate genes to gain better understanding of the temporal dynamics of gene regulatory mechanisms that contribute to priming-induced beneficial effects. In the long term, our study will likely provide important insights into how hormetic stress promotes stress resilience and health.

147 **Large Scale Analysis of Early Lethal Phenotypes** Jesse Mager University of Massachusetts

Early mammalian development requires that embryos accomplish specific milestones including implantation, axis specification and gastrulation. Since these events require precise timing of epigenetic regulation and complex morphogenetic movements for success, they are extremely vulnerable to genetic mutation and embryo lethality. Human data supports this idea with estimates as high as 80% of pregnancy losses occurring during the first trimester. Large scale projects designed to assess gene function in model organisms such as the Knock Out Mouse Project (KOMP) have also highlighted the stringent requirement of these early stages where ~12% of all null alleles result in lethality prior to E9.5. We have analyzed more than 200 novel early lethal single-gene knockouts produced by the KOMP. Assessment of such a large cohort of lethal alleles produced on the same genetic background, allows us to define specific phenotypic bottlenecks as well as predict essential gene networks. Rather than a distribution of phenotypes, we find the majority of homozygous mutant embryos have one of two specific phenotypes – a failure to implant or a failure to initiate gastrulation. Our data suggest that distinct mechanisms limit progression through each of these two stages. We propose that implantation failures occur due to molecular and/or transcriptional programing defects while gastrulation failures result from deficiencies in proliferation and/or cell number. Our findings serve as a guide for the analysis of early phenotypes and may inform the study of development of other eutherian mammals including humans.

148 **A cellular shearing mechanism important for dynamic tissue shape changes and cell differentiation** Liyuan Sui, Christian Dahmann TUD Dresden University of Technology

Dynamic changes in three-dimensional cell shape are important for tissue form and function. In the developing *Drosophila* eye, photoreceptor differentiation requires the progression across the tissue of an epithelial fold known as the morphogenetic furrow. Morphogenetic furrow progression involves apical cell constriction and movement of apical cell edges. Here we show that cells progressing through the morphogenetic furrow move their basal edges in opposite direction to their apical edges resulting in a cellular shearing movement. We further demonstrate that cells generate at their basal side oriented, force-generating protrusions. Knockdown of the protein kinase *Src42A* or photoactivation of a dominant-negative form of the small GTPase Rac decrease protrusion formation. Impaired protrusion formation stalls basal cell movement and slows down morphogenetic furrow progression and photoreceptor differentiation. This work reveals a cellular shearing mechanism important for the generation of dynamic tissue shape changes and cell differentiation.

149 **The Nucleus as a Barrier to Epithelial Cell Shape Changes** Rashmi Budhathoki[1], Noah deLeeuw[2], Liam J Russell[3], Dinah Loerke[2], James Todd Blankenship[1] [1]Department of Biological Sciences, University of Denver, [2]Department of Physics and Astronomy, University of Denver, [3]Department of Biological Sciences, Department of Physics and Astronomy, University of Denver

Planar polarized cell intercalation is a topology-driven event that brings tissue-scale changes for convergent extension movements during embryonic development in *Drosophila*. Here, we explore if the nucleus, as the largest organelle in a cell, poses a significant mechanical barrier to cells attempting to change shape and intercalate to achieve optimal body-axis elongation. We find that two central mechanisms allow nuclei to accommodate these stresses: 1) nuclear deformation and 2) the dispersion of nuclei to alternate planes along the apical-basal axis. We first tested the effects of restricting nuclear deformability with kugelkern disruptions. Embryos with “non-deformable” nuclei displayed a major shift towards the second mechanism; i.e., a more dynamic redistribution in apical-basal axis. Although this redistribution allowed cells to deform and still participate in cell intercalation, epithelial regularity was deeply compromised. We next tested embryos that possessed

"non-dispersible" nuclei by inhibiting microtubule polymerization. Interestingly, this caused a jamming phenotype in which nuclei became locked in the same apical-basal plane and cell intercalation was completely halted. Then, we asked what would happen in epithelia in which nuclei are both non-deformable and non-dispersible. In these circumstances, nuclei engaged in a tug-of-war to occupy the same z-plane in the absence of nuclear deformation and cell intercalation was again completely disrupted. These results suggest that the nuclear dispersion pathway is crucial for effective tissue elongation and guided by microtubules. To explore more on how this largest membranous organelle is being trafficked in apical-basal direction, we hypothesize three possible mechanisms: (a) motor protein-mediated transport, (b) microtubules pushing at actin cortex, and/or (c) nuclear translocation via microtubules of non-centrosomal origin. Our preliminary results suggest that dynein motors are necessary for centrosomal association with nuclei but are not the primary drivers of nuclear dispersion at this stage. Inhibiting CLASP, a microtubule plus end binding protein that has been known to interact with the actin cortex, caused loss of nuclear anchoring resulting in mispositioning of nuclei. These results suggest effective tissue elongation requires apical-basal trafficking of nuclei, which is promoted by microtubules that are anchored at the apical cortex.

150 **Positioning of the *Drosophila* hematopoietic niche** Kara Nelson, Stephen DiNardo University of Pennsylvania

Niches regulate stem cell behavior and are often found in specific locations within the tissues they support. How that position is established, and whether the specific positioning of a niche affects its function is unknown. I investigate formation of the *Drosophila* hematopoietic niche, the Posterior Signaling Center (PSC), which signals to hematopoietic progenitors to instruct their maintenance or differentiation into mature hemocytes. PSC positioning occurs during embryogenesis: PSC cells are specified laterally, then migrate to the dorsal surface of the embryo, where they ultimately reside, coalesced at the posterior of the hematopoietic gland. I live-imaged PSC migration which revealed that a muscle, visceral mesoderm (vm), moves dorsally alongside the PSC. We hypothesized that vm guides PSC positioning. Mutants that lack vm had mis-positioned PSCs, suggesting vm is required for PSC formation. Vm is known to express the secreted ligand, Slit, which is important for morphogenesis of other tissues. To test whether Slit is the vm signal that guides PSC positioning, I analyzed embryos where Slit signaling was eliminated only from vm. PSCs were mis-positioned, indicating vm-derived Slit signaling is required for PSC formation. To test whether Slit acts on the PSC as a directional guidance cue or a cue that allows it to be competent to migrate, I overexpressed Slit in a tissue nearby the forming PSC: the heart. These PSCs were mis-positioned, suggesting that Slit instructs PSC formation via directional guidance. Future experiments will test whether embryos with mis-positioned PSCs exhibit aberrant stem cell regulation.

151 ***C. elegans* Hedgehog-related Proteins are Apical Extracellular Matrix Components** Nicholas Serra, Chelsea Darwin, Meera V Sundaram University of Pennsylvania Perelman School of Medicine

C. elegans has no ortholog of Hedgehog or most of its canonical signaling partners. However, the *C. elegans* genome does encode 60 proteins thought to be evolutionarily related to Hedgehog (Hh-r) across four families: Warthog, Groundhog, Groundhog-like, and Quahog. The Patched related (PTR) gene family, the Hedgehog receptor in other systems, has undergone similar expansion in nematodes. Because most Hh-r genes lack obvious signaling roles or loss of function phenotypes, the purpose of these expansive protein families remain mysterious.

We report that endogenously tagged Hh-rs from the Groundhog-like and Warthog families are components of apical extracellular matrices (aECMs), specialized protective layers at the surface of all epithelial cells. Some of these proteins are continuously expressed, cell-specific components of cuticle. Other Hh-rs localize to the pre-cuticle, a transient aECM that precedes and patterns the cuticle proper. FRAP analysis indicates that these Hh-rs are stably incorporated into matrix. WRT-10, a newly identified pre-cuticle Hh-r, is required for normal formation of alae, acellular cuticle ridge structures, demonstrating a functional role in aECM organization. The lipocalin LPR-3 and patched related protein PTR-4 are each required for WRT-10's endocytosis during pre-cuticle clearance. Our emerging evidence suggests that Hh-rs are structural components of aECM, and that PTRs exert temporal control over Hh-r localization in a tissue specific manner. These findings suggest that *C. elegans* Hh-r proteins have diversified to meet the nuanced demands of aECM construction and function in diverse tissues. In addition, the aECM could serve as a reservoir for Hh-rs with proposed signaling roles, linking aECM remodeling and cell signaling.

152 **The Reissner's Fiber assembles downstream of local motile cilia activity to control body morphology** Elizabeth A Bearce[1], Sam Bertrand[2], Sophie Fisher[3], Zoe H Irons[2], Adamend Freda[3], Dan T. Grimes[3] [1]IMB, University of Oregoon, [2]Institute of Molecular Bio, University of Oregon, [3]University of Oregon

The linear body axis is a key feature of vertebrate life. It forms during embryogenesis based around the notochord and later the spine, a rigid but flexible column of vertebrae. We study the mechanisms that give rise to proper spine morphology. In zebrafish, generation and maintenance of the body and spine requires the function of motile cilia. Zebrafish embryos lacking cilia motility fail to undergo axial straightening and instead exhibit a curved early body axis and, later, scoliosis-like spinal

curves. Remarkably, we found that returning cilia motility to mutants allows the self-correction of body and spine morphology, suggesting a mechanism in which abnormalities are sensed and corrected. Motile cilia are required to assemble the Reissner Fiber (RF), an extracellular thread in the central canal composed of the protein Scospondin which we hypothesize to be important for the "sense-and-correct" mechanism. However, while Scospondin mutants do demonstrate morphological defects, we currently do not understand how RF forms downstream of motile cilia or how RF functions to promote a linear spine. To address this, we are performing live-imaging of cilia motility and RF in a variety of mutant backgrounds. This revealed that dynamic, rostral-caudal RF formation is dependent on local cilia motility in the central canal. When cilia are immobilized, knot-like RF structures form and eventually, the RF breaks down into diffuse monomers, something which correlates with the onset of spine dysmorphology. Our imaging has also revealed that central canal neurons uptake RF material in mutants that develop spinal curves. We propose that neuron-RF interactions, followed by endocytosis of RF material, function as a sensory system that allows the large-scale shape of the body and spine to be sensed, facilitating a path to maintain and correct organ morphology during growth, aging, and disease.

153 **Mechanosensitive live cell extrusions shrink the gut following food withdrawal** Aparna Sherlekar[1], Yu-Han Su[2], Samantha Thomas[1], Keerthana Yellapragada[2], Lucy E O'Brien[2], Emile G Magny[1] [1]MCP, Stanford University School of Medicine, [2]Stanford University School of Medicine

Many mature organs shrink when functional demand is reduced. This adaptive resizing response serves to optimize the organism's physiological fitness. However, the cellular processes that enable mature organs to shrink are largely unknown. Investigating the adult Drosophila gut, we discover that the gut's epithelial lining culls ~50% of its cells within two days of food withdrawal through massive cell extrusion. Live, in vivo imaging shows that during steady-state organ renewal, extrusions are slow and limited to late-stage apoptotic cells. By contrast, during starvation-induced shrinkage, extrusions are rapid and include both early-stage apoptotic cells and–strikingly–non-apoptotic cells. Comparative 4D analysis of single extrusion events suggests that the biophysical force required for cells to eject from the gut epithelium is weaker during shrinkage, which enables cells to readily exit. Screening mechanosensitive ion channels, we identify that the Pickpocket sodium channel may enforce the biophysical suppression of cell extrusions at steady-state and must be inhibited for starvation-triggered extrusions. Our results suggest that food withdrawal alters the mechanical state of the gut epithelium to permit low-energy extrusions, thus tuning cell number and organ size to fit functional demand. This work identifies a new role for cell extrusion in adaptive resizing of a mature epithelial organ and demonstrates that extrusions are a key mechanism for adaptive shrinkage.

154 **Control and Sensing of Spatially Patterned Embryonic Genome Activation Is Essential for Early Development** Wenchao Qian[1], Hui Chen[2], Matthew C Good[1] [1]Cell and Developmental Biology, University of Pennsylvania, [2]Biological Sciences, University of South Carolina

A watershed event in early development is the transition from maternal to zygotic control of embryogenesis, requiring awakening of the embryonic genome and widespread nascent transcription in a process termed zygotic genome activation (ZGA). In some embryonic models, ZGA onset is not spatially uniform, instead blastomeres display a temporally graded pattern of zygotic gene expression. How an embryo determines the precise timing of genome activation is an area of intense research investigation. Recently, we demonstrated in the *Xenopus laevis* model blastula that large-scale ZGA onset occurs in a spatiotemporal manner, contingent on embryonic blastomeres reaching a threshold cell size or DNA:cytoplasm ratio. Because amphibian embryos contain gradients of cell sizes along the animal-vegetal axis at the blastula stage, we wondered whether this cell size gradient specifies the pattern of ZGA and is essential for development. To address this question, we constructed a device to modulate the timing of cell division spatially within the blastula and reverse the gradient of blastomere sizes. Intriguingly, this manipulation is sufficient to reverse the graded patterning of ZGA onset. Functionally, alteration to the pattern of ZGA onset gradients causes embryo death, dose-dependent on the extent to which ZGA is delayed in the presumptive ectoderm. ZGA gradient reversal along the animal-vegetal activates an embryo quality control pathway, triggering apoptosis. Imposition of ZGA onset gradient along any other embryonic axes has no impact. Additionally, embryo death can be recapitulated by blocking ZGA in presumptive ectoderm. Co-injection with inhibitors of apoptosis prevent embryo death, and the data are consistent with a model in which embryos require spatially ordered expression of an anti-apoptotic program to fulfill the early quality control pathway. This work provides insights on mechanisms that regulate the timing of genome activation and how cell size is leveraged to coordinate tissue patterning and development.

155 **A genetic model for metazoan programmed DNA elimination** Thomas Dockendorff, Brandon Estrem, James Simmons, Maxim Zagoskin, Jansirani Srinivasan, Ryan Oldridge, Mehwish Iftikhar, Vincent Terta, Abigail West, Joe May, Jianbin Wang The University of Tennessee, Knoxville

Programmed DNA elimination (PDE) is a notable exception to the paradigm of genome integrity. In metazoa, PDE often occurs coincident with germline to somatic cell differentiation, resulting in reduced somatic genomes. Despite comprehensive

descriptions of the sequences lost in many metazoa, the mechanisms and consequences of PDE are still lacking due to the limited tools available in existing models. Recently, we established a functional and genetic model for PDE in the free-living Rhabditidae nematode *Oscheius tipulae*, a family that includes *Caenorhabditis elegans*. We identified a conserved motif, named Sequence For Elimination (SFE), for all 12 break sites on the six chromosomes at the junction of retained and eliminated DNA. CRISPR-edited SFE mutants exhibit a "fail-to-eliminate" phenotype only at the modified sites. To address the consequences of failure to eliminate and assess the fate of the inadvertently retained DNA, we are characterizing the SFE mutants to identify phenotypes and molecular changes in RNA expression, small RNAs, and histone modification marks. We will also present our data on the sequence determinants and genomic position required for the function of SFEs and our efforts to explore intraspecies variations of PDE by building and comparing telomere-to-telomere genomes for divergent strains of *O. tipulae*. This newly established PDE model is promising to provide insights into the mechanisms and functional significance of PDE in a metazoan.

156 **Regulation and function of non-canonical cell cycles in *C. elegans* and human hepatocyte organoids** Ramon Barrull Mascaró, Gabriella Darmasaputra, Christa Jordan Ortiz, Matilde Galli Hubrecht Institute

Many plant and animal cells transition from canonical to non-canonical cell cycles during development, resulting in the formation of polyploid cells. Two types of non-canonical cell cycles exist: endoreplication, where cells increase their DNA content without entering M phase, and endomitosis, where cells undergo all phases of the canonical cell cycle except cytokinesis, leading to a 4N or binucleate cell. Although endoreplication has been extensively studied in plants and insects, much less is known on the regulation and function of endomitosis, which is the most common mode of polyploidization in mammals. To study how cells transition to endomitosis, and what the consequences are of polyploidy for cells and tissues, our lab makes use of two experimental systems: the *C. elegans* intestine, which undergoes endomitosis and endoreplication cycles during larval development; and human 3D organoid cultures of primary hepatocytes (hep-Orgs), where cells can undergo both canonical or endomitosis cycles *in vitro*. Using live-imaging, single molecule fluorescence in situ hybridization (smFISH) and RNA-sequencing we find that *C. elegans* intestinal cells and human hepatocytes undergo endomitosis by inhibiting different aspects of cytokinesis signaling. Despite variations in the mechanisms leading to polyploidy, we observe remarkable similarities in the consequences of polyploidization. In both *C. elegans* and hep-Orgs, elevated ploidy levels are associated with increased transcription, protein translation, and cellular volume. Notably, not all functions scale proportionally to ploidy. In *C. elegans*, we find that mRNA transcription increases significantly during the first two intestinal polyploidization cycles, but does not substantially increase in the cycles thereafter, when cells reach ploidies of 16N and higher. Conversely, 26S rRNA transcription more closely follows DNA dosage, and continues to increase at higher ploidies. Also in polyploid hepatocytes, we find that ribosome biogenesis and protein sythesis scale with ploidy, unlike what has been reported in cells that are forced to become polyploid by induced cytokinesis failure. Thus, naturally occurring polyploid cells may have evolved mechanisms to specifically increase protein biosynthesis, which would explain why polyploidy is common in highly metabolically active tissues. Taken together, our work is shedding light into the regulation and function of somatic polyploidy, providing insights into how and why cells modify their cell cycles during multicellular development.

157 **Actin nucleator Arp2/3 promotes efficient DNA repair during *C. elegans* meiosis** Jordan E Brown, Diana E Libuda Biology, University of Oregon

Faithful genome inheritance in most sexually reproducing organisms requires meiosis, a specialized form of cell division that generates haploid gametes. To ensure accurate chromosome segregation, developing germ cells required the formation and repair of DNA double strand breaks (DSBs) using homologous recombination to generate crossovers. Chromosomes that fail to establish crossovers segregate incorrectly can lead to aneuploidy, a common cause of birth defects and miscarriages in humans. Recent work has found that the cytoskeletal protein actin is essential for the successful execution of meiosis in mammalian oocytes, and actin filament bundles can be detected in budding yeast nuclei during the specific stage of meiosis when active DSB repair is occurring. Notably, several studies established that actin facilitates DSB repair via homologous recombination in mitotic cells. While the presence and timing of nuclear actin in meiotic cells suggests a role for actin in meiotic DSB repair, a functional role for actin in meiotic DSB repair has not been established. Using a combination of live imaging, high-resolution deconvolution microscopy, and genetics, our data indicate that the presence of actin and actin nucleation by the Arp2/3 protein complex facilitates efficient DSB repair during meiotic prophase I in *Caenorhabditis elegans* oocytes. Using a conditional loss of Arp2/3 in the germline, we find that efficient meiotic DSB repair requires actin nucleation by the Arp2/3 protein complex. Specifically, our data indicate loss of Arp2/3 leads to an increase in the number of DSBs and a delay in DSB repair in the germ line. Coinciding with the impact on meiotic DSB repair, the conditional loss of Arp2/3 in the germline also causes a drop in fertility, which suggests Arp2/3 assists in the formation of viable oocytes. Further, we find the timing of chromosome pairing is altered upon loss of Arp2/3, thereby indicating that stable and accurate homologous chromosome associations that are critical for meiotic DNA repair via homologous recombination may require actin. Overall, this study is illuminating the specific role of actin and the cytoskeleton during meiotic prophase I of germ cell

development to protect the genome from instability.

158 **Transcriptional regulators modulate DNA damage response** Gonen Memisoglu[1], Nevan Krogan[2], James E Haber[3], Alexander J Ruthenburg[4] [1]Molecular Genetics and Cell Biology, The University of Chicago, [2]Department of Cellular and Molecular Pharmacology, University of California San Francisco, [3]Department of Biology and Rosenstiel Basic Medical Sciences Center, Brandeis University, [4]Department of Molecular Genetics and Cell Biology, The University of Chicago

We performed an unbiased genetic screen to identify novel factors that modulate the DNA damage response in budding yeast. This analysis revealed a previously uncharacterized genetic interaction between the essential DNA damage kinases and the cyclin kinase module (CKM) of the Mediator of transcription; a large protein complex that functions as a general transcriptional factor. We find that all subunits of the CKM, as well as CKM's kinase activity are critical for cell cycle re-entry following a DNA break, whereas the rest of the Mediator is largely dispensable. CKM mutants do not significantly impair DNA repair by homologous recombination; however, confer sensitivity exclusively to double strand break inducing reagents, suggesting that CKM specifically impinges on DNA damage response to double strand breaks.

We also discovered that a single DNA break is sufficient to significantly downregulate global transcription in a CKM-dependent manner. Our ChIP analyses show that in response to DNA damage, CKM downregulates global transcription by antagonizing general transcription machinery. Notably, akin to CKM mutants, RNA polymerase II mutants with faster elongation rates are also defective in cell cycle re-entry following a DNA break. Conversely, slowing down the RNA polymerase II elongation rescues the mitotic re-entry defect of CKM mutants, illustrating that CKM-dependent transcriptional regulation is essential for proper DNA damage signaling and mitotic re-entry.

Our preliminary data illustrate that the pharmacological inhibition of CKM in mammalian cells also lead to a hyperactive DNA damage signaling. Taken together, our results point to an evolutionary conserved role for CKM in DNA damage signaling and mitotic re-entry.

159 **Defective crossover homeostasis compromises chromosome segregation in aged mouse spermatocytes** Lakshmi Paniker[1], Vindhya Kilaru[1], Rhea Kang[1], Melissa Frasca[1], Suhani Bhakta[1], Isabella Ferranti[1], Ericka Humphrey[1], Francesca Cole[2] [1]The University of Texas MD Anderson Cancer Center, [2]University of Texas MD Anderson Cancer Center

Homologous recombination during meiosis generates crossovers, which are required to connect homologous chromosomes for their accurate segregation into gametes. Meiotic recombination is initiated by double-strand breaks (DSBs) generated by the SPO11 transesterase. In multiple organisms, a critical buffering mechanism, crossover homeostasis, maintains crossover numbers despite variation in DSB numbers. In mouse spermatocytes, both natural and genetic variation of early recombination intermediates on the order of ±25% is efficiently buffered to maintain crossovers at the expense or profit of an alternative repair product, noncrossovers. We have found in aged mouse spermatocytes, that DSB numbers are reduced by ~20%. However, unlike adult or juvenile mouse spermatocytes, crossovers in aged spermatocytes bear the brunt of reduced DSBs with 40% of spermatocytes lacking connection between homologs, in particular the sex chromosomes. Further reducing meiotic DSBs by removing a single copy of *Spo11* leads to 60% of aged spermatocytes lacking crossovers between homologs. We also show that there are two noncrossover pathways, an early acting pathway that likely contributes to chromosome pairing and a later acting pathway that likely serves as a reservoir for crossover control. Consistent with this model, in adult mouse spermatocytes, only the latter noncrossover pathway is utilized as a buffering reservoir when DSB numbers are reduced. In aged mouse spermatocytes, crossover homeostasis is compromised and cannot favor crossovers at the expense of this later noncrossover pathway. As a consequence, chromosomes frequently mis-segregate in aged mouse spermatocytes. Intriguingly, studies to determine whether older human spermatocytes have higher risk of chromosome mis-segregation have been controversial. What these studies lack is an investigation of the frequency of meiotic DSBs in the individuals tested for chromosome segregation. The frequency of DSBs is highly variable both between cells, but also between individuals. We will present our latest investigations into the mechanisms of crossover homeostasis and how this process is compromised with age in spermatocytes.

160 **Recombination rate plasticity depends not only on environmental stressors, but also genomic context and genetic background** Laurie S Stevison[1], Natalia Rivera-Rincon[2], Ulku Huma Altindag[1], Taylor Novak[2] [1]Auburn University, [2]Biological Sciences, Auburn University

For over a century, scientists have known that meiotic recombination rates are plastic, thus varying considerably among individuals depending on environmental and/or physiological stress. Through an examination of genome wide variation in recombination rate plasticity in ~3k progeny, we identified a global signature of increased recombination rate across the genome in *D. pseudoobscura* (p=0.02). Despite this global signal, we found variation in the degree of plasticity across chromosomes, with the strongest plasticity on chr4 and the least on chrX. Further, we found variation in the direction of

change at a fine scale (~100kb resolution), with many regions significantly reduced under heat stress. The distribution of crossovers was also significantly altered with increases in crossover number per chromosome (p=0.007) and the proximity of double crossovers (p=0.0023), suggesting that heat stress breaks down both crossover assurance and interference. Comparing these short-term changes to long term changes in recombination rate in close relatives, we showed that intervals with greater divergence in their recombination rates, both within and between species, had greater plasticity than intervals with conserved recombination rate over evolutionary time. In addition to recombination rate, we investigated individual stress response measures such as physiology and reproductive output, which vary by stock, sex and among individuals. Based on these results, we have begun to investigate the role of genetic background on recombination rate plasticity. Specifically, using the DGRP and a visible marker stock of *D. melanogaster*, we have shown recombination rate to be significantly reduced due to increased caloric density (p=3.6e-5) and low oxygen environments (p=1.9e-4), particularly in stocks that show increased physiological sensitivity to these stressors. In combinations of stress factors, we found an increased recombination rate regardless of genetic background (p=3.2e-3). Interestingly, we found that underlying differences among stocks in recombination rate under control conditions significantly impacted plasticity across treatments. Together, this work in different Drosophila species reveals recombination rate to be variable due to both differential exposure and susceptibility to environmental stress with complicated interactions. This outcome has important implications for how changes in the environment can interplay with genetic background to alter the landscape of genetic variation in natural populations.

161 **Aneuploidies of Specific Chromosomes upon Loss of the Spindle Checkpoint Protein Bub3 in Budding Yeast** Pallavi Gadgil[1], Olivia Ballew[2], Soni Lacefield[1] [1]Geisel School of Medicine at Dartmouth, [2]Indiana University

Aneuploidy generally has deleterious effects to growth rates of cells. However, studies have shown that cells with mutations in certain regulatory genes can gain specific chromosomes to provide survival and growth benefits. We found that loss of spindle checkpoint protein Bub3 results in cells that are aneuploid for specific chromosomes. In budding yeast, the spindle checkpoint is not essential, but loss of the checkpoint genes *BUB3* and *BUB1*, have a higher probability of chromosome mis-segregation than loss of *MAD1, MAD2,* and *MAD3*. This difference is likely due to the role of Bub3 and Bub1 in helping recruit the chromosome passenger complex to the inner centromere, which is involved in correction of improper kinetochore-microtubule attachments. The prior assumption was that these aneuploid cells would eventually be lost in the population since they have growth defects. However, we observed that although all chromosomes had an equal probability of mis-segregation initially, the *BUB3*-deleted (*bub3*Δ) haploid cells only maintained specific chromosomes and these chromosomes persist through many generations. Thus, we tested two types of survival advantages. First, we asked if evolved cells had a growth advantage compared to the non-evolved cells. We found that some evolved strains did have an advantage, but most did not. Next, we asked whether the *bub3*Δ cells gain and maintain specific chromosomes because the elevated expression levels of a subset of genes from the gained chromosomes can compensate for the loss of Bub3 or because the gain of these chromosomes is tolerated. We tested individual genes on each chromosome and performed a scan of all the genes on one of the upregulated chromosomes to determine if their increased expression would prevent aneuploidy of that chromosome. We found that the increased expression of multiple genes can help prevent aneuploidy of one of the specific chromosomes. Overall, our results suggest that the increased expression of certain genes, by maintaining aneuploidy of specific chromosomes, may be advantageous to cells lacking Bub3.

163 **Genetic basis of lethal mutations and how they persist in nature** Sarah Marion, Mohamed Noor Duke University

For nearly a century, evolutionary biologists have observed chromosomes which cause lethality when made homozygous persisting at surprisingly high frequencies (>25%) in natural populations of many species. These curious findings provide a challenging question fundamental to understanding natural fitness variation: given the extreme deleterious nature of lethal mutations, why do they appear at such high frequencies? Although most research on this question dates to the 1930's, we still know remarkably little about the genetic basis of naturally occurring lethal mutations or what evolutionary forces create their frequencies. Before even attempting to explain why lethal alleles are so common in nature, it is crucial to understand their genetic underpinnings. Decades of research has assumed lethal chromosomes are due to single locus, loss-of-function mutations, but this *has never been directly tested*. Even less is known about the classes of genes in which lethal mutations occur or their distribution throughout the genome.

We are using wild *Drosophila melanogaster* to determine the genetic basis of lethal mutations. *D. melanogaster* were collected from a natural population in Durham, North Carolina, and balancers were used to isolate ~300 independent samples in which chromosome 2 is homozygous lethal. By crossing a set of deficiency lines (that span over 70% of the second chromosome) to each lethal line, we have mapped hundreds of lethal lesions to defined regions of chromosome 2. Fine scale mapping and sequencing results provide novel characterization of naturally occurring lethals, including a nonsense mutation in the *drosha* gene. While this is evidence that at least some lethal alleles are single locus, we also provide novel evidence that most or all lethal alleles are single locus using Poisson-based mapping expectations.

We present the most extensive mapping study of naturally occurring lethal alleles ever conducted. Our results are the first direct demonstration for a single locus, loss-of-function mode of action of lethal mutations and provide sequence-level characterization of naturally occurring lethal alleles. Beyond underlying genetics, we discuss potential evolutionary force(s) responsible for the maintenance of lethal mutations and future analyses of our mapping and sequence data that will provide modern insight to a century-old question.

164 **Comparison of mutation rate in different ploidy states of the fission yeast *Schizosaccharomyces pombe* as a test of the drift-barrier hypothesis** Kevin Bao, Rutuja M Gupte, Nathaniel Sharp Genetics, University of Wisconsin, Madison

Why is there variation in mutation rate between organisms? Organisms have all evolved accurate DNA replication and repair and yet mutations still occur and at different rates. One theory is that selection has limited ability to eliminate weak mutator alleles which are maintained by drift. This is termed the drift-barrier hypothesis. Tests of this hypothesis have largely relied on comparing measurements of effective population size (N_e) and mutation rate, but getting accurate measurements of N_e is difficult. Alternatively, we propose that measuring cell-type specific mutation rates can be used as a test of the drift-barrier hypothesis. If a given organism rarely undergoes cell division in one cell context, then selection will have less opportunity to optimize the replicative machinery that applies only to this context. One example of such a context is ploidy. Thus, yeast species offer an excellent opportunity to test this hypothesis because they can grow as either haploid or diploid cells. Prior work in the diplontic budding yeast *S. cerevisiae* corroborates this idea. Using mutation accumulation (MA) – a method using repeated bottlenecks to render selection ineffective and allow spontaneous mutations to accumulate – the per site mutation rate was found to be 40% higher in haploids than in diploids for single nucleotide mutations (SNMs). The higher mutation rate in the rare ploidy state is consistent with our prediction, but could alternatively be explained if haploidy is inherently mutagenic. To separate the effect of ploidy versus evolutionary history, we performed MA for over 1700 generations in the haplontic fission yeast *Schizosaccharomyces pombe*. After sequencing, we found that the SNM rate was 45% lower and the insertion/deletion (indel) rate was 40% lower in haploids than in diploids. This is consistent with the drift-barrier hypothesis because mutation rate was significantly higher in diploid *Sc. pombe* – the historically uncommon and theoretically less optimized cell state. Not only is this the first time that the diploid mutation rate of *Sc. pombe* has been measured, but also this lends support to the idea that cell environment matters for the evolution of mutation rate. This presents a novel approach to testing the drift-barrier hypothesis and will help resolve the controversy over the role of genetic drift in mutation rate evolution

165 **Selection can locally reduce deleterious germline mutation rates via the deposition of H3K36me3 and DNA methylation** Leandros Boukas[1], Afrooz Razi[2], Hans T. Bjornsson[3], Kasper D. Hansen[2] [1]Johns Hopkins University/Children's National Hospital, [2]Johns Hopkins University, [3]Johns Hopkins University/University of Iceland

Understanding whether selection on regional mutation rate modifiers has led to reduced mutation rates at regions where mutations are more likely to be deleterious is a long-standing problem in molecular population genetics. In recent years, the drift-barrier hypothesis has been proposed as a simple yet powerful argument which states that selection on local mutation rate modifiers is too weak to overcome drift in finite populations.

Here we revisit this question. We focus on characterizing trans-acting mutation rate modifiers that: a) preferentially affect the mutation rate of regions most important for fitness; b) affect the mutation rate of many such regions at once. Such modifiers occupy an intermediate place between global mechanisms (such as DNA repair variants) and entirely local effects, raising the intriguing possibility that selection on them could be effective in the face of drift. First, we show - using a dataset of 18,365 de novo synonymous mutations - that genes that are more intolerant to loss-of-function mutations in humans have lower coding mutation rates. We confirm this result by examining the synonymous substitution rate from a human-chimp comparative analysis, and further show that the relationship between loss-of-function intolerance and coding mutation rate disappears if one includes intronic mutations; this explains why it has been missed in previous studies. We then show that a histone modification, H3K36me3, is preferentially deposited at coding exons of loss-of-function intolerant genes, and is associated with a lower mutation rate at these exons.

To examine the effect of drift, we perform simulations in finite populations. Our simulations are based on the same model underlying the theoretical foundation of the drift barrier. We modify this model with a parameter that explicitly reflects the number of sites whose mutation rate is simultaneously affected. Using empirically estimated mutation rates and selection coefficients, we find that coding mutation rate modification via selection in favor of exonic H3K36me3 can overcome the drift barrier. We also establish a similar result for DNA methylation at promoters, showing that selection can act against promoter DNA methylation to reduce the mutation rate at CpG sites and thereby preserve promoter CpG islands. Of note, our simulations incorporate empirical estimates of the rate at which DNA methylation and H3K36me3 patterns change in the germline, via a human-chimp-rhesus comparative analysis which shows that these marks are extremely stable over time.

In summary, we find that mutation rate modification of coding sites and promoter CpG sites via selection in favor of exonic H3K36me3 and against promoter DNA methylation, respectively, is evolutionarily plausible. Our results provide insights into the evolution of germline mutation rates, and the interaction between mutation rates and gene regulatory systems.

166 **Natural selection causes association studies to prioritize variant specificity over variant impact** Hakhamanesh Mostafavi, Jeffrey Spence, Mineto Ota, Nikhil Milind, Tami Gjorgjieva, Jonathan Pritchard Genetics, Stanford University

Genome-wide association studies (GWAS) have long been used in an attempt to find variants and genes that strongly impact a phenotype of interest. Yet, GWAS hits can be difficult to interpret, and a large amount of phenotypic variance is often explained by variants with no obvious trait relevance. This raises the question of what variants are prioritized by GWAS. Here, we argue that association studies prioritize variants that are most specific to the trait under study --- not necessarily the variants with the largest effects. We show that this prioritization of specificity is a consequence of natural selection acting on several independent axes of phenotypic variation and variants having differing degrees of pleiotropy. We use this insight to compare the types of genes discovered by GWAS and rare variant burden tests. We also show how this view explains the observation that heritability is often strongly enriched in genomic regions that are specifically active in the trait-relevant cell-type even if the variants are not acting through obviously trait-relevant genes.

167 **The impact of sample design on the observed site frequency spectra and its relevance for the discovery of rare deleterious variants in natural populations and associations with phenotypic variation** Margaret C Steiner[1], Daniel P Rice[2], Arjun Biddanda[3], Marida Ianni-Ravn[1], Christian Porras[4], John Novembre[5] [1]Human Genetics, University of Chicago, [2]University of Chicago, [3]Johns Hopkins University, [4]Ichan School of Medicine, [5]Human Genetics, Ecology and Evolution, University of Chicago

A key question in any study design is whether it is advantageous to sequence as many individuals as possible in one geographic location (“narrow sampling”) or to sequence individuals from as many geographic locations as possible (“broad sampling”). Here we consider this question in the context of efforts to discover variants that affect phenotypic traits under stabilizing selection, such that rare variants are selected against. We ask: how does the spatial breadth of sampling impact the observed site frequency spectrum, and how does that in turn affect one’s power to make association with phenotypic trait variation? With such questions in mind, we introduce a novel theoretical framework to study the impact of spatial sampling design on the discovery of rare variants. In particular, we develop a population genetic model for the distribution of carriers of deleterious alleles in a structured population – accounting for dispersal, drift, selection, mutation, and uneven spatial sampling simultaneously. Analysis of our model suggests that, for a fixed sample size, broader sampling will result in the capturing of a greater number of deleterious variants, but each variant will be at reduced frequency within the sample; in contrast, narrow sampling yields fewer variants but each is found at higher frequency within the sample. To investigate how these different discovery profiles impact the power to discover associations to phenotypes, we then integrate our work with statistical genetic models to identify trade-offs for genome-wide association studies (GWAS) and burden test studies. We find that under our model, power to detect a single-variant association typically decreases for broader sampling – plausibly due to the dilution of allele frequencies in broad samples – unless variant effect size is above a critical value. However, power to detect gene-level associations using a burden test, which combines signals across many loci, increases for broader sampling. We validate the theory results using simulations and then explore whether the predictions of the theory are borne out in human whole-genome-sequencing data from gnomAD and the UK Biobank and using an example from *Arabidopsis thalania*. Overall, the results imply that, for samples with increased breadth, burden tests will be key to identifying phenotypic associations. We expect our analyses will help to inform a broader discussion regarding best practices for study design in the sequencing era, both in humans and a broader set of organisms. They also have relevance for understanding the site frequency spectra and biases introduced by sample design more generally.

168 **Background selection landscape in human genomes: insights from Ancestral Recombination Graph** Yun Deng[1], Rasmus Nielsen[2], Yun Song[2] [1]Computational biology, UC Berkeley, [2]UC Berkeley

Background Selection (BGS) has shaped the diversity patterns along human genomes with the effects of linkage. With development in BGS theory, people have been able to predict diversity level in human genome matching real data accurately. However, the prediction has worked mainly for large scale, often for 1Mb windows. Also, the region-specific background selection coefficients have not been explicitly inferred, because previously only diversity patterns on large scale were leveraged and the dimensionality is much lower than the number of selection parameters for conserved regions. Here we show that with Ancestral Recombination Graph (ARG) inference, we can look into much finer-scale diversity variations, which provides more information for learning the genome-wide selection landscape. Our results shed new lights on BGS analysis, which

provides a much-finer scale B-map and estimation of background selection in a region specific way along the genome, instead of summarizing as Distribution of Fitness Effects (DFE). The regions with inferred strong background selection align well with CADD tracts (inferred from cross-species conservation), while only using human population level data. We believe that applying this new method to different species will greatly benefit the understanding the sharing and variation of background selection along evolutionary history.

169 **The relationship between the mutation spectrum and the distribution of fitness effects across species** David Castellano, Justin Conover, Ryan Gutenkunst University of Arizona

Understanding the interplay between DNA mutation rates and fitness effects is a fundamental question in evolutionary biology. In this study, we investigate this relationship in three model organisms: humans, *Mus musculus*, and *Arabidopsis thaliana*, using polymorphism data from natural populations and germline mutations from several sources. Our analysis in *Arabidopsis* shows that the 3mer mutations with the highest mutation rates are more deleterious. In contrast, in humans and mice, we find that the most mutable nonsynonymous 3mer mutations have less deleterious effects. This pattern is particularly pronounced within non-CpG transitions in both humans and mice, and within non-CpG transversions in mice, suggesting that the mutation spectra in these two mammalian species tend to be less disruptive to fitness than the mutation spectrum in *Arabidopsis*. We suggest that the differences observed between *Arabidopsis* and mammals are likely due to sequence-dependent DNA repair enzymes. Without these enzymes, highly mutable nonsynonymous 3mer mutations are quickly removed due to their high mutation rates, unless they are kept by strong negative selection, resulting in the correlation we observed in *Arabidopsis*. In contrast, in humans and mice, these enzymes may exist and be under natural selection to reduce the impact of highly mutable 3mer mutations imposing a greater genomic deleterious burden, resulting in a distinct mutation spectrum. Our work highlights the contrasting relationship between mutation rates and fitness effects between these species and suggests the singular role of repair mechanisms in mammals.

170 **Environment-independent distribution of mutational effects emerges from microscopic global epistasis** Sarah Ardell[1], Alena Martsul[1,2], Milo S Johnson[3,4], Sergey Kryazhimskiy[1] [1]Ecology, Behavior and Evolution, University of California San Diego, [2]Platform Reagent Development Group, Illumia, Inc, [3]Integrative Biology, University of California Berkeley, [4]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory

Adaptive evolution can lead to profound changes in the phenotypes and behaviors of biological systems, sometimes with adverse and sometimes with beneficial consequences for human health, agriculture and industry. However, predicting these changes remains difficult. One major challenge is that how new mutations alter phenotypes and fitness of organisms often depends on the genetic background in which they arise (G×G interactions or “epistasis”), the environment (G×E interactions), or both (G×G×E interactions). Phenotypic predictions rely on coarse-grained “macroscopic” descriptions of these interactions, typically captured by the distributions of mutational effects. Several recent studies reported that the effects of many mutations on fitness scale with the fitness of the background genotype, a phenomenon termed “global epistasis”. However, how the patterns of global epistasis vary across environments is unknown and how global epistasis shapes the distributions of mutational effects is unclear.

To address these questions, we measured the effects of ~100 barcoded insertion mutations on growth rate in 42 strains of *Saccharomyces cerevisiae* in six laboratory environments. We find that the slopes of the relationship between the effects of mutations and background growth rate remain nearly invariant across environments, despite substantial variation in both the effects of individual mutations and background-strain growth rates. Instead, the environment tunes one global parameter, the background fitness at which most mutations switch sign, which we term “the pivot growth rate”. As a result, the distribution of mutational effects is remarkably predictable across genotypes and environments.

This apparent simplicity in how the effects of individual mutations and their distributions vary across genotypes and environments has potential implications for our understanding of evolutionary dynamics, phenotypic prediction and conservation biology. At a more fundamental level, it suggests that organism’s architecture imposes some as of yet uncharacterized constraints on the effects of mutations.

171 **Increasing undergraduate access to research with *C. elegans*** Joslyn Mills Biology, Bridgewater State University

Hands-on learning is essential for students to fully grasp complicated concepts, but a single exposure to a technique or idea during a semester is usually not enough to achieve long-term retention of understanding. Students must be engaged in interactive learning in order for them to more successfully retain the concepts, increase attention, and have meaningful experiences which leads to significant learning[1,2]. Further, success in the STEM fields correlates with exposure to research experiences before the period of attrition from the STEM majors[3,4]. With this approach in mind, I designed a Course-based

Undergraduate Research Experience (CURE) course to be offered to all Biology students that only need introductory biology to register. This course brings discovery-based research into the classroom to foster technical and conceptual learning, either as an introduction to or to increase retention of students in STEM[5] fields. It provides the hands-on laboratory and project design experience students need to become more competitive to join a research lab for their eventual thesis work; particularly for those students that have not had the advantage of working in a lab before (minorities, females, etc[6]). Students with prior experience in high school are typically favored, thereby limiting inclusivity and diversity. Therefore, the long-term goal of this course is to expose many students from a variety of backgrounds to research and help them meet curriculum requirements in support of the academic programs and associated labs to prepare them for future exciting STEM careers.

In the course, the students take on the role of a scientist, where they perform a reverse genetics screen in C. elegans to select a gene to explore for the rest of the semester. After selecting their gene, as a small group, they develop their own hypothesis and write and execute a proposal to test it using published literature to support their ideas, ending with a written report and oral presentation of their findings. This approach allows us to gather clues to what the function of a gene is, and we can further determine if this gene would be a potential target to design therapeutics to treat diseases. This CURE course is easily adaptable to be offered at other institutions, and has been executed in traditional (fully in-person), fully online, and hybrid formats with great success.

172 **Melanogaster: Catch The Fly!: A Citizen Science Network in Adaptation Genomics** Josefa González[1], Miriam Merenciano[2], Marta Coronado-Zamora[1], Ewan Harney[1], Irene González[2], Sònia Casillas[3], Roberto Torres[2] [1]Institut de Biologia Evolutiva (CSIC-UPF), [2]La Ciència Al Teu Món, [3]Universitat Autònoma de Barcelona

The citizen science project *Melanogaster: Catch The Fly!* (*MCTF!*) brings together high school teachers and students, from rural areas of Spain, Germany, Ukraine, Serbia, and Colombia, and their local communities, with scientists from all across Europe (and beyond), and communication experts to collaborate towards the advancement of frontier science. *MCTF!* offers citizens the possibility of actively participating in several research activities, thus providing them with a unique opportunity to learn about the scientific process, while they contribute to facilitate and expedite scientific research. *MCTF!* Also provides the participants the opportunity to increase their scientific, technological, and 4C capacities (collaboration, communication, creativity, critical thinking), through a hands-on project that empowers them as agents and enablers of a necessary global change.

In *MCTF!,* school teachers and students participate in collecting and classifying *Drosophila* species (fruit flies) that are then sequenced and analyzed to understand how organisms adapt to the environment. The data generated is shared through the European Drosophila Population Genomics Consortium (DrosEU). The activities of the *MCTF!* citizen science project also include: teacher training courses in genomics and science communication; the experimental validation of genetic variants using the PCR technique; bioinformatic analysis modules that introduce high-school and university students in big data analysis; and an activity based on the service and learning methodology that allow citizens to monitore the effects of climate change on local biodiversity.

173 **Mini-CURE molecular genetics lab exercises using *C. elegans* and toxicology** Julie Hall Biology, Lincoln Memorial University

Course-based undergraduate research experiences (CUREs) give students the opportunity to explore and investigate scientific topics within the structure of a classroom. Students are led through the development of research statements, execution of experiments, and analysis of data to foster an understanding of the scientific process. These mini-CURE lab exercises differ from traditional CUREs because they are only a few weeks in length. They bring in the learning objectives of the CURE without having to be invested in an entire semester or part of a bigger project allowing other topics to be covered within the lab course. These exercises are part of a 300-level molecular genetics lab course in which students choose a toxicant to investigate for two experiments using *C. elegans*. These can be adapted for a variety of courses and may be conducted with other model organisms. Lab 1 focuses on utilizing reverse transcription and PCR to analyze gene expression. Lab 2 is a design your own experiment where students pick an outcome (movement, growth, reproduction, or death) to observe after exposure to their toxicant or stressor of choice. Students work in pairs and begin by performing a literature search to pick a toxicant or stressor for the study and determine two concentrations to test for the experiments along with an outcome to test in Lab 2. Lab 1 allows students to learn RNA isolation, PCR, and gel electrophoresis while testing a hypothesis related to gene expression. Lab 2 provides more opportunities for students to have choice in which they explore an effect of the toxicant or stressor that interests them. Students present their research through the writing of an introduction, results, and discussion sections in the form of a journal style paper. Student choice with these two labs provides them with the opportunity to have ownership of the experiments. Students also practice skills of developing research statements and hypothesis through literature research and investigation. Although the design of this lab lasts a total of 6 weeks within a semester, modifications can be made to lengthen the time to focus more on student directed design or shorten the lab depending on the structure and time of the course.

These labs are reported as being a favorite within the course and students become invested in conducting the experiments because they got to choose what was being tested (toxicant) and what they wanted to test (outcome).

174 **A virtual lab meeting training program benefits both mentees and host labs** Katie Lotterhos[1], Moisés Bernal[2], Megan Phifer-Rixey[3], Torrance Hanley[4] [1]Northeastern University, [2]Auburn University, [3]Drexel University, [4]Sacred Heart University

Professional networks are critical for early career scientists. However, access to these networks is variable and inequitable, with greater barriers for students from systemically-marginalized groups, first generation college students, and students that attend institutions without extensive research programs. To help expand networks in evolution and marine sciences, the Research Coordination Network for Evolution in Changing Seas developed and ran a Virtual Lab Meeting Training Program. This program matched "Mentees," students at the undergraduate or graduate level, with "Mentors," postdoctoral researchers, research scientists, or professors, that shared their interests. Mentees then attended lab meetings virtually, with at least two meetings devoted to the Mentees professional development, and received a stipend after completing the program. After running for two academic years, Mentees and Mentors were surveyed to evaluate the program. Results indicated that the program was largely effective. Mentees reported that they gained knowledge of the hidden curriculum of research science and expanded their professional networks. Mentors found that Mentees brought new perspectives and knowledge and that participating required only a few extra hours of time over the course of the program. Greater diversity among Mentees than Mentors also suggests that this type of program has the potential to help increase participation of early career scientists from marginalized groups. Importantly, Mentee responses suggest that stipends are a crucial part of the program and should be considered for future programs. Overall, we found that this type of program can help support trainee development with little additional investment from Mentors, translating to benefits to the field.

175 **An undergraduate course at the intersections of genetics, disability studies, and gender and sexuality studies** Karen G Hales Biology, Davidson College

Undergraduate genetics courses and textbooks often use language frameworks that invalidate or "other" disabled people and LGBTQIA+ people, including those with differences of sexual development. Shifting to more inclusive paradigms within undergraduate biology education may improve retention in STEM of people with these identities, and may contribute to a future medical workforce better equipped to empower disabled and LGBTQIA+ people and treat them with empathy and dignity. After previously making incremental relevant shifts in my longstanding genetics courses, I report here the development and implementation of a novel undergraduate seminar course entitled "Genetics, Disability, and Gender Identities" that explores intersections of genetics with the scholarly fields of Disability Studies and Gender and Sexuality Studies. An initial focus on the eugenics movement sets the historical background, showing how concepts of normality and fitness intertwined with ableist, hetero- and cis-normative, and white supremacist social systems. The first half of the semester then weaves biological analysis of deafness, Down syndrome, autism, and psychiatric conditions with disability studies frameworks such as the medical vs. social models of disability, also incorporating first person perspectives from people with those identities. Using such frameworks, students critically assess assumptions in the biomedical literature about molecular treatments as well as assistive reproductive technologies. In the second half of the course, students explore ongoing research on the biology of sexual development, sexual orientation, and gender identity, from work on a variety of organisms to recent genome-wide association studies in humans. In parallel, students analyze public conversations about whether or not such research is helpful or harmful to the LGBTQIA+ rights movement, views on gender from sociological angles, implementation of sex testing in sports, and impacts on health care and public policy. Strongly positive student feedback on the course indicated that building bridges across scholarly areas is important for establishing a complete and inclusive view of biological topics that connect with marginalized identities.

176 **Development and Retention of Female Academics Via a Peer-led Book Club Intervention** Amanda L Zacharias[1,2], Deborah Y Kwon[2,3] [1]Cincinnati Children's, [2]Genetics, University of Pennsylvania Perelman School of Medicine, [3]Neuromuscular & Movement Disorders, Biogen

Purpose: In response to concerns from fellow postdocs that there was insufficient institutional and individual support to encourage women to remain in the academic "pipeline", we created a peer-led book club intervention for female graduate students and postdocs. This intervention has operated successfully for over 4 years with support from the Department of Genetics at the Perelman School of Medicine at the University of Pennsylvania.

Design: Membership expands widely beyond the Department of Genetics to anyone on the department seminar listserv and is composed of 56% postdocs, with a sizable international contingent. Books are nominated and ranked in preference by the membership; selections have ranged from self-help titles aimed at women in business targeting common issues (negotiation, Imposter syndrome), the history of women in science, to new research on biological gender differences. Meetings are

held monthly in several small groups that meet over lunch or dinner at a consistent time to foster group cohesion and accommodate different schedules. These meetings not only provide education and awareness on gender-related issues in science, but also offer a safe environment where female scientists are able to share personal and professional issues. Once a semester, a joint session is held to hear a female faculty describe their career path, which serves to provide relatable examples and opportunities for trainees to network with faculty. Experts from across the Penn campus have also been brought to lead interactive workshops on relevant topics.

Results: Based on Likert scale surveys of 52 participants over 4 years, 94% reported the group helped them feel part of the university community, 80% reported increased confidence, 75% reported the group had helped them identify solutions to problems in their professional life, and 98% would recommend participating in the group to other women. All women departing the group thus far (n=14) have continued their scientific careers in academia, pursuing graduate education, postdoctoral fellowships, and faculty positions, or have attained other academic positions of scientific leadership. These results indicate that an intervention of only 12 hours per year may have significant impact on retaining women in academic science. We believe a peer-led book club could be a valuable intervention for other under-represented groups because it provides community while teaching soft-skills.

177 **Nematode Hunters: an integrated approach combining science outreach, course-based undergraduate research, and mentored research to identify novel nematode viruses** Jessica N Sowa[1], Katherine Przeworski[2] [1]Biology, West Chester University of Pennsylvania, [2]Pennsylvania Leadership Charter School

C. elegans and other related free-living nematodes are a popular and powerful model system for studying the cell biology and evolution of host-pathogen interactions. However, only four viruses capable of naturally infecting *Caenorhabditis* nematodes have been identified, and of those only one (Orsay virus) infects *C. elegans.* This severely limits *C. elegans* as a model system for studying host-virus interactions specifically.

To search for novel nematode viruses capable of infecting *C. elegans*, we have taken a community-science approach to obtain a diverse sampling of wild-caught nematodes. The Nematode Hunters outreach program partners with 4th grade classrooms, allowing teachers to implement a week-long classroom module with all supplies provided by WCU. In this module, students learn about nematodes, collect samples from their local environment, identify samples containing wild nematodes, and ultimately submit the nematodes to WCU to be screened for intracellular infections. Data collected from pre and post surveys indicates that participation in Nematode Hunters leads to both an increased knowledge of how science is conducted and recognition that they have participated in the scientific process for 4th graders in the program.

Once received, samples are screened via co-culturing of wild nematodes with *C. elegans* expressing intracellular infection reporters. This screening is conducted by students enrolled in a course-based undergraduate research class. Using this approach we have evaluated over 500 wild nematode isolates sent in from both the Nematode Hunters program and other collaborators, identifying more than 200 potential intracellular infections. To prioritize potential viral infections, students in the course homogenized reporter-activated populations and passed the homogenate through a 0.22uM filter to size-exclude other pathogen types. Follow up testing is then conducted by students in the Sowa lab, including FISH staining to detect Orsay or other known nematode viruses and collection of samples for RNA sequencing.

178 **Enhancing biology education in Nigeria: use of Drosophila as an effective teaching and learning aid** Rashidatu Abdulazeez[1], Monsurat Titi Gbadamosi[2], Hammed Badmos[3], Muhammad Oyale Akhadelor[4], Dawoud Usman[5], Salisu Muhammad[1], Nurudeen Aliyu[6], Abubakar Ibrahim Mukhtar[7], Hassan Sani[8], Saadatu Idris[9], Racheal Henry[10], Usman Liman Gambo[11], Jamila Musa[12], Emmanuel Kayode Ajibulu[1], Felix Faruq Uduimoh[1], Dalhatu Mukhtar Shehu[1], Nuhu Muhammad Danjuma[13] [1]Zoology, Ahmadu Bello University, Zaria, [2]Vivian Therapeutics, [3]Cagan Lab, University of Glasgow, [4]Federal College of Forestry Mechanization, Afaka, Kaduna State, [5]Biomedical Research and Training Centre, Damaturu, [6]Steamledge Limited, Kano, [7]Anatomy, Ahmadu Bello University, Zaria, [8]Biochemistry, Federal University of Lafia, Nassarawa, [9]Biology, Ahmadu Bello University, Zaria, [10]Anatomy, Federal University Wukari, Taraba, [11]Microbiology, Ahmadu Bello University, [12]Intergrated science, Federal college of education, [13]Pharmacology and Therapeutics, Ahmadu Bello University Zaria

Conducted as a precursor to a three-day workshop for biology teachers-in-training (B.Sc. (Ed)/ B.Ed. college students), this survey featured participants selected from seven universities nationwide. This diverse cohort primarily consisted of females (54.5%), originating from underrepresented regions of the country, while males constituted the remaining 45.5%. Notably, a significant portion of the participants had previously attended privately owned secondary schools. The survey's outcomes unveiled resounding support for practical teaching methods as the most effective approach to imparting biology knowledge. An impressive 68.2% of participants concurred that hands-on activities significantly augment students' understanding of biology. Furthermore, participants expressed a strong inclination towards conducting practical classes, particularly on subjects

such as genetics and DNA extraction from cheek cells, contingent on the availability of requisite resources. A striking revelation was that a substantial 72.7% of the participants could not recollect encountering Drosophila, commonly known as the fruit fly, in their secondary school biology textbooks. Similarly, a discernible knowledge deficit was apparent concerning Drosophila melanogaster, with 22.7% of participants acknowledging limited familiarity, and the highest proportion (54.5%) admitting to having very little knowledge about the fruit fly's nervous system. Nonetheless, a remarkable 72.7% of the participants demonstrated a keen interest in integrating Drosophila into their science teaching, signifying the considerable potential of this model organism in shaping the future of biology education. These findings cast light on the preferences and preparedness of biology teachers-in-training, offering valuable insights into how practical and hands-on teaching methods can be leveraged to enhance the biology education experience.

179 **Investigating the mechanisms by which hunger modulates an animal's olfactory behavior** Roshni Jain[1,2], Eryn Slankster[3], Yihe Zeng[3], Kelsie Dreelan[3], Jingmin Lin[3], Juli Petereit[4], Dennis Mathew[2,3] [1]Biology, University of Nevada, Reno, [2]Molecular Biosciences Program, University of Nevada, Reno, [3]Biology, University of Nevada, Reno., [4]Nevada Bioinformatics Center, University of Nevada, Reno.

An animal's olfactory behavior is modulated by its hunger. In insects and mammals, hunger modulates the function of first-order olfactory sensory neurons (OSNs). Previous research from our lab and others have suggested that hunger-dependent modulation of OSN function is mainly mediated by insulin, an anorexigenic hormone. However, the mechanisms by which insulin modulates OSN function are unclear. This research aims to understand the molecular mechanisms by which insulin mediates the hunger-dependent modulation of OSN function and, thereby, the animal's olfactory behavior. The experimental plan takes advantage of the *Drosophila* larva as a model system. The premise is that cell-surface insulin receptors translocate to the nucleus, associate with promoters, and regulate gene expression. First, RNA sequencing analyses of larval OSNs revealed that OSNs with low insulin signaling (mimicking a hunger state) had lower expression levels of several genes involved in vesicular transport, including *Rab5*. Rab5 protein is found on synaptic vesicles and regulates protein transport. Next, immunocytochemistry analyses confirmed that insulin signaling regulates the Rab5-dependent transport of several synaptic proteins, including Domeless (Leptin homolog), to the OSN terminals. Finally, behavior analyses showed that disrupting Domeless expression at OSN terminals affected the larva's olfactory and feeding behaviors. Overall, these results reveal a previously undescribed insulin signaling mechanism in OSNs—vesicular protein transport. Our work provides a foundation for further analyzing the mechanisms by which insulin mediates the hunger-dependent modulation of an animal's olfactory behavior.

180 **Type 3 iodothyronine deiodinase (DIO3) controls the timing and stability of cone development in human retinal organoids** Christina McNerney[1], Robert J Johnston[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

How neuronal subtypes are generated at distinct times during human retinal development is poorly understood. To understand how developmental timing is regulated, we investigated the choice between blue and red/green cone photoreceptor fates in human retinal organoids. Blue cones are specified before red/green cones and thyroid hormone (TH) signaling promotes red/green cone fate. How this signaling mechanism is temporally regulated is not understood. Here, we find that DIO3, an enzyme that degrades TH, is a master regulator of cone developmental timing. The ratio of DIO3-expressing cells decreases over time to control the timing, quantity, and stability of S and M/L cone generation. Negative feedback at the cellular level ensures homeostatic levels of TH signaling. These mechanisms control the timing of cone development and the stability of cell fates, which ultimately yield reproducible ratios of cone subtypes in the human retina.

181 **Velvet Ant venom activates both insect and mammalian pain sensors through distinct mechanisms** Lydia J Borjon[1,2], Luana C de Assis Ferreira[2,3], Andrea G Hohmann[2,3], W Daniel Tracey[1,2] [1]Biology, Indiana University, [2]Gill Center for Biomolecular Science, Indiana University, [3]Psychological and Brain Sciences, Indiana University

The Scarlet Velvet Ant (*Dasymutilla occidentalis*) has an extremely painful sting which has been described as "Explosive and long-lasting, you sound insane as you scream. Hot oil from the deep fryer spilling over your entire hand." Prior studies have shown that the defensive venom is effective across vertebrates including mammals, birds, amphibians, and reptiles. This leads to the hypothesis that the venom may target an evolutionarily conserved pain pathway. To test this, and to determine the venom's pain-causing mechanism of action, we applied venom to *Drosophila* larvae in a fillet preparation, and quantified neuronal activity using calcium-imaging with genetically encoded GCaMP6f. We found that velvet ant venom specifically activates larval nociceptors (cIV da neurons), without activating other sensory neurons (such as cIII da neurons). We tested candidate target channels whose expression is specific to nociceptors. Pickpocket (Ppk) and Balboa (Bba) are members of the DEG/ENaC and ASIC channel superfamily and are necessary for mechano-nociception in fly larvae (Zhong et al., 2010). RNAi against *ppk* or *bba* in cIV da neurons prevents activation by venom. The presence of both Ppk and Bba subunits are required for proper localization to sensory dendrites and the channel subunits likely form heterotrimers (Mauthner et al., 2014). We found that co-expression of Ppk and Bba in cIII da neurons is sufficient to cause these neurons to be strongly activated by

venom. However, expression of Ppk or Bba alone does not cause activation, providing evidence for the first time that Ppk/Bba heteromers form functional channels. *Dasymutilla occidentalis* venom is composed of 24 peptides whose amino acid sequences have been determined (Jensen et al. 2021). Testing each of these chemically synthesized peptides, we have identified noxious components of the velvet ant venom proteome. A single peptide is responsible for fast-acting nociceptor activation through Ppk/Bba channels. 5 other peptides activate neurons more generally, at a slower time scale and at higher concentrations. Behavioral experiments in mice indicate that the latter non-specific peptides trigger robust pain behaviors, but the peptide targeting the fly channels does not. This suggests a potent insect-specific component of the wasp venom, and we found that velvet ants can successfully defend against predation by praying mantis (*Tenodera sinensis*) using its venomous sting. Combined, our results suggest that the venom cocktail of velvet ants has diverse peptides that act across phyla to activate pain sensing neurons.

182 **Identifying cellular and molecular regulators of acoustic startle sensitivity** Kimberly Scofield[1], David Christopher Cole[2], Sruti Bontala[2], Sureni Sumathipala[2], Jacob Deslauriers[2], Kurt Marsden[2] [1]Biological Sciences, North Carolina State University, [2]North Carolina State University

Sensory over-responsiveness occurs when an individual is unable to ignore irrelevant stimuli, and it is often a debilitating component of neuropsychiatric disorders. To understand the mechanisms that enable appropriate sensory filtering, we study the acoustic startle response in larval zebrafish, a conserved defensive response that allows organisms to quickly escape from danger. If this response threshold is too low, however, the organism will overreact to innocuous stimuli. Through a forward genetic screen, previous work found that nonsense mutations in *Cytoplasmic FMRP Interacting Protein 2* (*cyfip2*) cause acoustic startle hyperresponsiveness. It is not known, though, whether this is due to enhanced detection of auditory stimuli or reduced filtering of sensory input. To address this, we measured the activity of hair cells using vital dyes and found no difference in activity of either otic vesicle or lateral line hair cells between *cyfip2* mutants and siblings. We also established a transgenic line that expresses the excitatory opsin CoChR in the auditory nerve, enabling us to directly activate the auditory nerve. If the hyper-responsiveness in *cyfip2* mutants is driven by enhanced detection, then directly exciting the auditory nerve will abolish it. Our data show that we can reliably elicit kinematically stereotyped startle responses with blue light that are distinct from light-driven behavioral responses. CoChR-expressing *cyfip2* mutants were not hyperresponsive to optical stimuli, but they were also not hyperresponsive to acoustic stimuli. Instead, we found that CoChR-expressing wildtype larvae were hypersensitive to acoustic stimuli compared to non-transgenic siblings, indicating that CoChR expression likely alters auditory nerve function. Startle responsiveness of *cyfip2* mutants was unaffected by auditory nerve CoChR expression, however, suggesting that auditory nerve neurons may already be hyperexcitable and could thus be a site of *cyfip2*-mediated startle threshold regulation. We will complement this optogenetic approach by measuring auditory nerve activity using the calcium indicator jGCaMP8m. We are also developing a transgenic line to allow us to express Cyfip2 in specific cell populations to determine where it is acting in the acoustic startle circuit. In total, these experiments will allow us to better understand the genetic and neural circuit mechanisms that control sensory filtering, providing key insight into sensory processing disorders.

183 **How flies and vector mosquitoes sense heat and humidity (and you)** Paul Garrity[1], Rachel Busby[1], Willem Laursen[1], Tatevik Sarkissian[2], Shruti Shankar[1], Ruocong Tang[1] [1]Biology, Brandeis Univ, [2]Biology, Brandeis University

Temperature and humidity are ubiquitous environmental variables with major effects on the survival and reproduction of insects like flies and mosquitoes. Temperature is a fundamental property of matter that affects all aspects of animal physiology, and animals must sense and respond to temperature to maintain body temperatures compatible with survival. Humidity reflects the concentration of water vapor in the air, and monitoring humidity helps insects avoid dehydration. In addition to supporting homeostasis, temperature and humidity sensation also play critical roles in helping disease-spreading vector mosquitoes (which transmit devastating diseases like malaria, dengue and zika) locate and bite humans based on the heat and humidity we emit. In addition, humidity sensation helps female vector mosquitoes locate water sources for egg laying --- this is a critical step in their reproduction, as mosquito larvae are aquatic. Thus, identifying and characterizing the molecular and cellular mechanisms by which insects sense and respond to temperature and humidity has potential relevance for understanding insect physiology as well as for vector control efforts.

In prior work in *Drosophila*, we have found that several classes of molecular receptors participate in mediating responses to temperature and humidity. These receptors include the Transient Receptor Potential (TRP) channel TrpA1, the Gustatory Receptor Gr28bD, and several Ionotropic Receptors (IRs), including Ir21a, Ir25a, Ir40a, Ir68a and Ir93a. Here we will present recent work in *Drosophila* that combines molecular genetics, physiology and behavior to identify a class of molecular receptor (and a set of neurons that express this receptor) that acts as a previously unappreciated mediator of temperature sensation and thermosensory behavior in the adult fly. In addition, we have previously found that IR family members initially identified in *Drosophila* have key roles in heat and humidity sensing in the malaria vector *Anopheles gambiae* and the arbovirus vector *Aedes aegypti*. Here we present recent work in *Aedes aegypti*, establishing that different IR family members make

distinct, but overlapping contributions to host seeking, blood feeding and oviposition site seeking behavior. Together, these IRs, and the neural circuits that rely on them, combine to allow mosquitoes to respond to a wide variety of host-derived cues, allowing them to hunt for blood meals effectively under a wide variety of environmental conditions.

184 **Neurexin (*nrx-1*) isoforms differentially mediate multiple foraging circuits and behaviors in *C. elegans*** Michael P Hart, Mara H Cowen, Brandon L Bastien Genetics, University of Pennsylvania

Some of the most conserved behaviors across animal phylogeny are feeding-related and involve responses to food and nutrients. Foraging behaviors range from searching for food to more complex social strategies (social vs. solitary foraging). The nematode *C. elegans* has robust behavioral responses to food, including multiple foraging behaviors and strategies, many with well-defined neurons, circuits, and signaling pathways involved. We used automated imaging platforms to assay multiple foraging behaviors in individual or populations of young adult *C. elegans*. Using longitudinal and unbiased quantification of behavior, we identified that the conserved synaptic adhesion molecule neurexin/*nrx-1* is required for *C. elegans* food search and social foraging strategies. Food search requires the cooperative function of both the short and long isoforms of *nrx-1* to regulate octopamine signaling, response, and octopamine neuron synaptic connectivity. Social feeding behavior requires the long isoform of neurexin in two pairs of glutamatergic sensory neurons (ADL and ASH), where it regulates connectivity of just the ASH neurons. Glutamate signaling from both ADL and ASH neurons is required for social foraging behavior, where we observe increased synaptic glutamate synaptic release in social animals compared to their solitary counterparts (which is independent of *nrx-1*). We report independent roles for the canonical partner of neurexin, neuroligin/*nlg-1*, in both food search and social foraging behaviors. These studies uncover molecular and circuit mechanisms that contribute to multiple foraging behaviors, including highly conserved genes.

185 **Defining neuronal extracellular vesicle (EV) subtypes by single-EV molecular analysis** Inna Nikonorova[1], Elizabeth A. desRanleu[1], Joshua Saul[2], Jonathon Walsh[2], Katherine A. Jacobs[2], Juan A. Wang[2], Maureen Barr[2] [1]Genetics, Rutgers University, [2]Rutgers University

Cilia are present in most cells in the human body, including the brain. Ciliopathies often present with neurological manifestations, such as intellectual disability, agenesis of the corpus callosum, hydrocephalus, intracerebellar cysts, and polymicrogyria. The underlying mechanisms through which cilia dysfunction leads to these profound defects in neurodevelopment remain enigmatic.

Polycystins are ubiquitous and essential ciliary proteins. Organ-specific knockout of polycystins leads to major dysfunctions via yet unidentified molecular cascades. Polycystins are highly expressed in the brain, and their role in the nervous system is beginning to be understood. In zebrafish, polycystins function in the cilia of CSF-contacting neurons of dorsolateral and ventral origin that regulate spine posture. Globally, loss of polycystin function in vertebrates leads to randomization of left-right body plan during embryogenesis with polycystins acting through EVs shed from the nodal cilium. Whether polycystins act via EVs in the nervous system of vertebrates remains to be discovered. Emerging evidence suggests that abnormal EV signaling within the brain may contribute to neuronal degeneration.

In *C. elegans*, polycystins function in the cilia of male-specific sensory neurons that direct mating behavior. These conserved polycystins are shed from the ciliary tip into the environment for animal-to-animal communication. The signaling mechanism and cargo of the neuronal polycystin-carrying EVs remain unknown.

Here, we use proximity labeling, super-resolution microscopy, and genetic perturbations in *C. elegans* to define polycystin-associated EV cargo. We have identified and validated TRAFs (tumor necrosis factor TNF receptor-associated factors), three transmembrane lectins with dorsoventral specialization, and a novel transmembrane protein that resembles an ion channel as cargo of polycystin-carrying EVs. We discovered that all newly identified partners of the polycystin complex required polycystin-1 for their loading to ciliary EVs. Disruption of polycystin-1 lead to the ciliary release of polycystin-2 EVs, which lack polycystin-associated transmembrane components and TRAF signaling adaptors. These findings suggest that the loading of cargo to ciliary EVs follows a regulated hierarchical process and demonstrates how a single genetic perturbation can profoundly affect the composition of a specific type of EV, impacting neuronal communication mediated by these vesicles.

186 **Neuromodulation of serotonin drives presynaptic plasticity underlying reward learning** aaron stahl[1], Valentina Botero[1], Seth Tomchik[1,2,3] [1]Neuroscience and Pharmacology, University of Iowa, [2]Pediatrics, University of Iowa, [3]Iowa Neuroscience Institute, University of Iowa

Associative learning involves temporally-specific actions of multiple monoaminergic neurotransmitters including serotonin (5-HT). Olfactory associative learning drives presynaptic plasticity in the release of acetylcholine (ACh) from mushroom body Kenyon cells (KCs). These changes in ACh release systematically vary across the longitudinal axonal compartments of the

KCs. Each of these compartments innervates unique downstream mushroom body output neurons (MBONs). In addition, each compartment receives heterosynaptic input from different sets/combinations of monoaminergic neurons. These monoaminergic neurons modulate the synaptic plasticity between KCs and MBONs. Serotonergic neurons innervate the KC compartments, and KCs express multiple serotonin receptors. Serotonergic neurons exert strong behavioral effects on olfactory classical conditioning, though their role in modulating the compartmentalized synaptic plasticity driven by reward learning is not known. Here we investigate the role of 5-HT in driving reward-associated presynaptic plasticity in KC compartments. We paired *in vivo* imaging of ACh release across the MB γ lobe with appetitive conditioning and conditional RNAi-mediated knockdown of 5-HT receptors in the KCs. Conditional knockdown of two different 5-HT receptors in adult mushroom body Kenyon cells altered the odor-evoked release of ACh across γ lobe compartments following appetitive conditioning, systematically shifting baseline odor-evoked ACh release and abolishing the conditioning-induced changes in ACh release across compartments. This loss of plasticity correlated with loss of behavioral learning – appetitive conditioning was impaired following the knockdown of different 5-HT receptor subtypes. These data suggest that layered signaling via monoaminergic pathways modulates baseline ACh release and drives plasticity underlying learning.

187 **A Notch signal required for a morphological novelty in *Drosophila* has antecedent functions in genital disc eversion** Donya N Shodja[1], William J Glassford[2], Sarah J Smith[3], Mark Rebeiz[4] [1]Biological Sciences, George Washington University, [2]Columbia University, [3]Yale, [4]University of Pittsburgh

A fulfilling explanation for the origin of morphological novelties requires a concrete understanding of the developmental program controlling the novelty as well as the pre-existing molecular foundation it was built upon. We therefore must uncover how key regulators of the program evolved expression associated with the novelty, how new genetic programs emerged downstream, and how these circuits relate to any ancestral roles that predated the new structure's emergence. Developmental signaling pathways that are conserved throughout animal development have been frequently implicated in the evolution of novelties. Here, we demonstrate that a Notch signaling center required for the posterior lobe, a morphological novelty on the male genitalia of *Drosophila melanogaster,* had ancestral roles fundamental to the proper formation of the entire adult male genitalia. We identified transcriptional enhancers of the ligand *Delta*, which allowed us to track the evolutionary history of this signaling center. Surprisingly, our analysis reveals that the signaling center becomes active days before the posterior lobe forms, serving an early-acting role necessary for genital disc eversion (Epper, 1983) – the process in which the epithelium underlying genital structures turns inside out. We provide a likely mechanism by which Delta contributes to genital eversion through a network of apical extracellular matrix, which also became integrated into the novel posterior lobe program. This work demonstrates that novelties may be formed in the context of already complex developmental processes, by appending new roles to pre-existing signals.

188 **Decoding developmental signaling using molecular optogenetics in zebrafish** Catherine E Rogers[1], Allison J Saul[2], Leanne E Iannucci[2], William K Anderson[2], Selvaraj Velanganni[2], Patrick Müller[3], Katherine W Rogers[2] [1]Cell, Molecular, Developmental Biology, and Biophysics, Johns Hopkins University - NIH partnership, [2]NICHD, National Institutes of Health, [3]Universität Konstanz

Cell fate decisions during embryogenesis are influenced by signaling levels, dynamics, and combinations. To experimentally manipulate these signaling features and determine how they are decoded in zebrafish embryos, we are developing a suite of orthogonal optogenetic tools that activate BMP, Nodal, and FGF signaling in response to blue or red light. We demonstrate that the blue light-responsive optogenetic tools activate specific pathways with blue, but not red, light exposure. In addition, we are quantifying the tools' on/off kinetics and light intensity-dependence and working to generate transgenic zebrafish harboring optogenetic activators. To create orthogonal tools to simultaneously activate two pathways independently, we are systematically testing different red light-responsive construct designs in zebrafish embryos. In ongoing experiments, we are applying these tools together with RNAseq to identify transcriptome-wide changes in gene expression in response to acute, optogenetically delivered signaling pulses during early development. These optogenetic tools provide a powerful platform to investigate how embryonic cells decode signaling levels, dynamics, and combinations. Funding: NIH Intramural ZIAHD009002-01 to KWR.

189 **WheresWally: a bioinformatic pipeline for rapid mutation mapping using whole genome sequencing** McKenna Feltes[1], Sofia Angel[1], Aleksey Zimin[2], Steven Salzberg[2], Steven Farber[1] [1]Biology, Johns Hopkins University, [2]Biomedical Engineering, Johns Hopkins University

Forward genetic screening is a powerful, unbiased approach that has led to numerous discoveries across many fields of biology. Historically, identification of the causative mutation was achieved by recombinant mapping, a time consuming process which relies on a mapping outcross. More recently, the development of affordable and rapid sequencing platforms has enabled "mapping-by-sequencing" approaches that utilize RNA or DNA sequencing datasets to generate lists of candidate mutations located in genomic intervals linked to the phenotype. Gene candidates can be tested using reverse genetic approaches such as

CRISPR editing. “Mapping-by-sequencing” represents a massive leap forward in mutation mapping, however, when genomic intervals and candidate lists are large, gene testing becomes intractable. To address this limitation, we have developed an analysis pipeline, WheresWally, which combines modern bioinformatic tools with classical recombinant mapping to rapidly identify causative mutations generated by forward genetic screening. We have designed WheresWally using ENU mutagenized zebrafish, but it can be adapted to suit other organisms and/or mutagens. The pipeline defines the linkage block by identifying regions of high SNP homozygosity in the mutant genomic sequence which is generated by whole genome sequencing (WGS) of a pooled mutant bulk. The linkage block is compared to the wildtype block to identify segregating small nucleotide polymorphisms (SNPs) and insertions and deletions (indels). Indels serve as markers which can be used to identify recombinants which further narrow the linkage block and candidate gene list. Compared to traditional recombinant mapping markers, these indels are present at a higher density and are specific to the bulk analyzed, allowing for better mapping resolution without an outcross. Using this approach we have mapped 6 novel alleles generated from an ongoing zebrafish mutagenesis screen designed to identify genes controlling lipid flux. In the era of CRISPR knockout reverse genetics, WheresWally aids in the identification of hypomorphic point mutants generated by forward genetic mutagenesis screens which remain powerful tools for the study of gene function and mechanism.

190 **Ecolocator: A supervised machine learning model for location and climate-of-origin prediction** Jordan Rodriguez[1], Andrew Kern[1], Richard Cronn[2] [1]Biology, University of Oregon, [2]United States Forest Service

As the Earth’s climate changes, nearly every organism on the planet will shift its geographical range in response. For ecologically and economically important species, it is vital that scientists and managers be able to make informed decisions about future suitable environments for populations. In previous work we have shown that supervised machine learning methods are capable of predicting location of origin from genomic variation using georeferenced training data. However, the spatial genetic variation signal that we are capturing may be the result of both neutral processes (e.g. drift) and local adaptation. Reasoning that the connection between genotype and environment mediated by local adaptation might be discernable from neutral variation, we introduce Ecolocator, a deep learning method that jointly predicts location of origin as well as environmental variables of that location. Ecolocator trains on a dataset of genotypes with known geographic location and associated climate variables such that it can predict the climate-of-origin and geographic location based on the genotype of an unlabeled sample. To validate our approach, we applied our method to the coastal variety of the most important timber species in the world, *Pseudotsuga menziesii var. menziesii* (Douglas fir), which previously has been shown to have strong local adaptation. Using a preliminary dataset of ~340 georeferenced genotypes and associated climate variables for those locations from ClimateNA, we are able to predict where an individual genotype might be best suited to given environmental conditions throughout the range. Our open-source tool clarifies the biological implications of the connection between environmental variation and genetic variation so that informed conclusions regarding the conservation, adaptation, and resiliency of this species can be made.

191 **ProteinCartography: Comparing proteins with structure-based maps for interactive exploration** Prachee Avasthi, Brae M Bigge, Mert Celebi, Jase Gehring, Erin McGeever, Atanas Radkov, Dennis A Sun Arcadia Science

Proteins are a fundamental building block of all organisms. Comparing proteins across different organisms can allow us to identify conserved functions, as well as instances of evolutionary innovation. Historically, such analysis was performed by comparing protein sequences. However, recent advances in protein structure prediction (AlphaFold, ESMFold) and comparison (Foldseek) have also enabled the systematic comparison of protein structures between organisms. Building on these tools, we developed “ProteinCartography,” a pipeline which generates interactive visualizations of protein structure comparisons. ProteinCartography searches available sequence and structure databases for matches to user input proteins, compares all hits, and clusters proteins based on their structural similarity. It reports results through interactive visualizations that summarize protein metadata including functional annotations, taxonomy, structure prediction quality, and other metrics, allowing users to rapidly explore protein space. Using ProteinCartography, scientists are able to develop specific hypotheses about their proteins of interest using a comparative framework with greater ease, clarity, and depth than conventional search approaches.

192 **Leveraging genome-scale molecular networks from multiple species using machine learning to translate gene-level data and knowledge across species** Christopher A Mancuso[1], Kayla A Johnson[2], Alexander McKim[3], Keenan Manpearl[2], Renming Liu[3], Hao Yuan[4], Julia Ganz[4], Ingo Braasch[4], Arjun Krishnan[2] [1]Biostatistics and Informatics, University of Colorado Anschutz Medical Campus, [2]Biomedical Informatics, University of Colorado Anschutz Medical Campus, [3]Computational Mathematics, Science and Engineering, Michigan State University, [4]Integrative Biology, Michigan State University

Research organisms are critical for studying complex mechanisms underlying human biology and disease. Nevertheless, thousands of genes in humans and model organisms remain experimentally un(der)-characterized in terms of their roles in cellular pathways, cell type/tissue function, phenotypes, and diseases. This significant gap in knowledge is a major barrier to

delineating, in an unbiased manner, the best genes in different model systems to study multifactorial biomedical phenomena and translate the findings back to human biology. Varying evolutionary distances and lineage-specific genetic and functional changes further compound this barrier.

To overcome this barrier, we are developing advanced machine learning (ML) approaches that take advantage of millions of biomolecular interactions in the form of genome-scale gene/protein networks spanning multiple species (e.g., human, mouse, zebrafish, fruit fly, roundworm, and yeast). Specifically, we have developed a new ML framework called GenePlexusZoo that casts molecular networks from multiple species into a single "functional" space where genes in similar network neighborhoods, within and across species, are close to each other. Multiple rigorous evaluations demonstrate that this multi-species network representation improves the ability to predict novel genes associated with pathways, traits, and diseases within a single species and across species, even in cases where the inter-species correspondence is undetectable based on shared orthologous genes.

Now, we have expanded the capabilities of GenePlexusZoo to handle highly multifactorial, polygenic conditions (e.g., Autism Spectrum Disorder) with the goal of identifying a collection of model phenotypes (even from different organisms) that can together recapitulate the complexity of each condition. After such phenotypic deconvolution of a complex disorder, our approach prioritizes specific genes in each model system pertinent to each phenotype, which can be followed-up experimentally.

Our framework is a significant improvement over existing methods because it has desirable properties that are lacking in existing methods: the ability to handle networks from multiple species, incorporate many-to-many orthology information, and generate a network representation that is reusable across different types of and newly-defined prediction tasks. GenePlexusZoo is available as an open-source software and an interactive webserver for all researchers to use and build on.

193 **Biomarker Curation, Integration, and Data Model Development** Daniall Masood[1], Raja Mazumder[2], Jeet Vora[2], Sean Kim[2], Karina Martinez[2] [1]Department of Biochemistry and Molecular Medicine, George Washington University, [2]Department of Biochemistry and Molecular Medicine, George Washington Univeristy

In the pursuit of evaluating disease and medical intervention outcomes, biomarkers emerge as pivotal tools for researchers and clinicians. Biomarker data is extensive, but disorganized over a vast number of papers and resources. This project endeavors to establish a standardized biomarker definition and consolidate diverse biomarker data under a unified data model.

Our biomarker definitions and model follow the FDA-NIH Biomarker Working Group's definition: "a characteristic measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention". Ongoing efforts are underway to develop a biomarker-centric view of data within the Biomarker Partnership, funded by the Common Fund Data Ecosystem, an NIH initiative. The data model currently encompasses 'core' and 'contextual' biomarker data, forming the foundation for biomarker definition.

Core data is essential to the model and must be fulfilled for a biomarker to be added to the data model (e.g. biomarker, assessed_biomarker_entity, condition). Contextual data (any extra data related to a biomarker) originates from various partner resources within the Biomarker Partnership, integrated into the data model framework. Any absent data is addressed through inference, guided by biomarker-centric rules. Biomarker curation follows this standardized data model, drawing from both manual curation of biomarker-related papers and automated extraction from public resources that have biomarker or biomarker-related data. The curated data includes essential biomarker information (e.g., biomarker entity, change, disease), ensuring accessibility for incorporation into the data model. Contextual data, curated from diverse partner resources, is integrated into the data model, and supplemented through inference if necessary. This approach solidifies the groundwork for an expansive biomarker knowledge graph.

GlyGen (glygen.org), a glycoscience knowledgebase, is a current example of how biomarker data is harmonized, integrated, and represented. These biomarkers are associated with glycan and protein identifiers. While currently focusing on glycan and protein biomarkers, GlyGen lays the framework for how biomarker data can become integral part of biological knowledgebases.

The culmination of biomarker data curation and the standardized data model promises unprecedented accessibility and search efficiency for biomarkers thereby improving their findability, accessibility, interoperability, and reuse.

194 **A genome-wide genetic screen uncovers determinants of human melanogenesis** Vivek K Bajpai Chemical, Biological & Materials Engineering, University at Oklahoma

Melanin, an inert biopolymer, is responsible for skin, hair and eye pigmentation. Consequently, genetic variants affecting melanogenesis underlie diversification of human species into distinctly pigmented subpopulations. Melanin production also contributes to the natural homeostasis of tissues that produce it, and dysregulation of these pathways can lead to slew of human pathological conditions affecting the skin (e.g., vitiligo and melasma), eyes (age related macular degeneration) and ear (sensorineural deafness). Melanin is typically produced by melanocytes, which originate from the neural crest and reside in the epidermis, where they transport melanin filled vesicles (a.k.a. melanosomes) to the surrounding keratinocytes, resulting in skin pigmentation. Importantly, the number of melanocytes and their anatomical location do not vary among humans, only varying melanogenesis determines the human pigmentation diversity. The core biochemical machinery that regulates melanin synthesis has been known for a long time and further key insights have come from mapping genes involved in hypo- and hyper-pigmentation diseases and from genome-wide association studies (GWAS) of normal-range skin and hair color variation in human populations. However, current candidate GWAS variants can explain only a fraction of skin color variation in humans, suggesting that many loci controlling pigmentation remain undiscovered. To fill this knowledge gap, we leveraged the light scattering properties of melanin and performed a genome-wide CRISPR-Cas9 screen to systematically discover genes driving human melanogenesis. We first demonstrated a tight correlation between melanin content of pigment cells and their light scattering properties as measured as side scatter parameter (SSC) by flow cytometry using pigment cell lines and human pluripotent stem cell derived model of melanogenesis. We next exploited this relationship to directly survey genes involved in melanogenesis through a genome-wide CRISPR screen. We uncovered 169 functionally diverse genes converging on melanosome biogenesis, endosomal transport and gene regulation, of which 135 represent novel associations with pigmentation. In agreement with their melanin-promoting function, the majority of screen hits are upregulated in melanocytes from darkly pigmented individuals. We further unravel functions of KLF6 as a transcription factor regulating melanosome maturation and pigmentation in vivo and of the endosomal trafficking protein COMMD3 in modulating melanosomal pH. Our study reveals a plethora of melanogenesis regulators, with broad implications for human variation, cell biology, and medicine.

Reference: Bajpai et al., Science 2023.

195 **Update on the Alliance of Genome Resources** Paul W Sternberg[1], Carol Bult[2], Alliance of Genome Resources[1] [1]California Institute of Technology, [2]The Jackson Laboratory

The Alliance of Genome Resources (aka, the Alliance) is a consortium of model organism knowledgebases (MODs) and the Gene Ontology Consortium (GOC). The mission of the Alliance is to support comparative genomics as a means to investigate the genetic and genomic basis of human biology, health, and disease. To promote sustainability of the core community data resources that make up the Alliance, we have implemented and maintain an extensible “knowledge commons” platform for comparative genomics built with modular, re-usable infrastructure components that can support informatics resource needs across a wide range of species. These include JBrowse, InterMine, BLAST, Textpresso, a common Community Forum, and other features we are developing. We will briefly describe our progress towards harmonizing information from SGD, WormBase, FlyBase, ZFIN, Xenbase, MGD, RGD and the GO Consortium resources, storing that information in a useful form available by software, download, or via our web portal at www.AllianceGenome.org. Our goal is to provide the Union of the data and features of these resources (as opposed to the Intersection), and we fully expect to provide better service to all our communities. The individual MODs will continue to exist and in addition to continuing to curate information from the scientific literature, large scale experiments, and submissions from individual investigators , they will innovate methods for data analysis and display and interact with their respective communities. In 2022, the Alliance was recognized as a Core Global Biodata Resource by the Global Biodata Coalition, a step that we hope will allow us to engage international funding to further enhance this resource.

196 **Investigating differences in mitotic checkpoint strength in the early *C. elegans* embryo** Priscila A Medrano Gonzalez, Imge Ozugergin, Abigail R Gerhold Department of Biology, McGill University

The mitotic checkpoint preserves genome stability by inhibiting the anaphase-promoting complex and delaying mitotic exit until all chromosomes are attached to the mitotic spindle via kinetochore-microtubule attachments. Despite its central role in preventing chromosome segregation errors, checkpoint strength (i.e., the ability to block mitotic exit in the presence of unattached kinetochores) varies between species, cell types, and developmental stages. The reasons for this variation are poorly understood. In *C. elegans* embryos, checkpoint strength correlates with cell size. Checkpoint strength increases as cell size decreases during early embryonic cleavage divisions and when cell size is reduced experimentally in 2-cell stage embryos, using RNAi treatments that affect embryo size. We have found that cells from small 2-cell stage embryos tend to have a stronger checkpoint than similarly sized cells produced during cleavage, suggesting that cell size is not the sole determinant of checkpoint strength in *C. elegans* embryos. To better understand what underlies this difference, we characterized the expression levels, localization, and dynamics of endogenous, GFP-tagged mitotic proteins, including the core checkpoint proteins MDF-1/Mad1, MDF-2/Mad2, BUB-3, SAN-1/Mad3, over the first 5 rounds of embryonic cleavage divisions. We found

that expression of MDF-2/Mad2 increases during this window of development, likely via *de novo* translation of maternally supplied transcripts, such that smaller cells later in development have a higher concentration of MDF-2/Mad2 than larger cells at earlier embryonic stages. While the expression of MAT-2/Apc1, an essential scaffolding subunit of the anaphase-promoting complex, also increases, the rate of increase is significantly outpaced by that of MDF-2/Mad2. As activated MDF-2/Mad2 is a rate-limiting factor in anaphase-promoting complex inhibition and checkpoint activity, we predict that increased MDF-2/Mad2 expression contributes to increased checkpoint strength as cell size decreases during development. We are currently testing this prediction, as well as assessing whether similar differences affect checkpoint strength in small 2-cell stage embryos. These results suggest that developmental factors act in parallel to cell size to determine checkpoint strength in *C. elegans* embryos, and that checkpoint protein expression may be a general determinant of checkpoint strength.

197 **Developmental phenotypes caused by the inappropriate inheritance of histone methylation require Polycomb Repressive Complex 2** Zaynab A Massenburg, Sydney Morgan-Benitez, Brandon Carpenter Kennesaw State University

Histone methylation is a post-transcriptional modification to the N-terminal tails of histone core proteins that regulates DNA accessibility, and consequently, gene expression. At fertilization, histone methylation must undergo maternal reprogramming to reset the epigenetic landscape in the new zygote. During maternal reprogramming of histone methylation in *C. elegans*, H3K4me is removed by the H3K4 demethylase, SPR-5, and H3K9me is subsequently added by the histone methyltransferase, MET-2. Recently, we demonstrated that SPR- 5; MET-2 maternal reprogramming antagonizes the H3K36 methyltransferase, MES-4, which maintains a transcriptional memory of a subset of germline genes between generations. Maternal loss of SPR-5 and MET-2 results in ectopic expression of MES-4 germline genes in somatic tissues and a severe developmental delay. Recent work from the Strome Lab shows that MES-4 repels the activity of the Polycomb Repressive Complex 2 (PRC2), which includes the H3K27 methyltransferase, MES-2, at germline gene loci in the germline. These findings prompted us to examine whether PRC2 complex-dependent H3K27me3 may contribute to the developmental delay in *spr-5; met-2* mutants by concentrating ectopic H3K36me3 at germline gene loci in the soma. To test this possibility, we knocked down MES-2 using RNAi and found that the developmental delay in *spr-5; met-2* mutants is completely rescued. By performing RNA-seq and ChIP-seq, we further demonstrate that knocking down MES-2 rescued the ectopic transcription of MES-4 germline genes and reduced the ectopic H3K36me3 at these loci in *spr-5; met-2* mutant somas. Together, these data suggest that the PRC2 Complex contributes to a soma-to-germline transition and developmental delay in *spr-5; met-2* mutants and provides mechanistic insight into how highly conserved histone modifying enzymes cooperate during development to establish germline versus somatic cell fates.

198 **The CYK4 GAP domain controls contractile ring assembly and dissolution by regulating the cortical targeting of centralspindlin** Aleesa Schlientz[1], Sebastian Gomez-Cavazos[2], Kian-Yong Lee[2], Pablo Lara-Gonzalez[2,3], Arshad Desai[2], Karen Oegema[2] [1]School of Biological Sciences, University of California, San Diego, [2]University of California, San Diego, [3]University of California, Irvine

During cytokinesis, an equatorial contractile ring constricts to partition the cell contents. Contractile ring assembly requires active GTP-bound RhoA generated by the guanine nucleotide exchange factor ECT2. The centralspindlin complex, composed of two molecules of kinesin-6 and two molecules of CYK4, is the major ECT2 activator during cytokinesis. Centralspindlin is phosphorylated by Polo-like Kinase 1 (PLK1) at the central spindle during anaphase and diffuses to the adjacent plasma membrane where it activates ECT2. The N-terminal half of CYK4 is the primary target of PLK1 and engages with ECT2 to activate it, likely by relieving ECT2 autoinhibition, whereas the C-terminal half of CYK4 contains a C1 domain, which contributes to plasma membrane targeting, and a GTPase-activating protein (GAP) domain predicted to interact with a Rho family GTPase. The function of the CYK4 GAP domain has been unclear. Here, we show that the CYK4 GAP domain functions with the adjacent C1 domain to target centralspindlin to the cell cortex. Using an assay we developed to monitor cortical recruitment of centralspindlin during cytokinesis in the one-cell *C. elegans* embryo, we show that RhoA and the GTPase-binding interface of the CYK4 GAP domain are both essential to recruit centralspindlin to the equatorial cortex. Comparison of a CYK4 mutant that disrupts the RhoA binding interface of the GAP domain to a catalytic mutant (R459A) that selectively disrupts the ability of the GAP domain to convert bound RhoA-GTP to Rho-GDP revealed striking differences. In a mutant background partially compromised for RhoA activation, preventing RhoA binding inhibited furrowing, whereas the catalytic mutant was largely able to complete cytokinesis. In addition, whereas the RhoA-binding mutant reduced centralspindlin recruitment to the equatorial cortex, the catalytic mutant did not. The catalytic mutant additionally seemed to prevent the normal dissolution of cortical centralspindlin and contractile ring components at the end of cytokinesis. These results lead us to propose a model in which cortical recruitment of centralspindlin by the CYK4 GAP domain is central to a feedback loop in which generation of active RhoA at the cell cortex drives further centralspindlin recruitment and RhoA activation to drive rapid contractile ring assembly. As the contractile ring disassembles, conversion of RhoA-GTP to RhoA-GDP by the CYK4 GAP domain is important to ensure timely dissolution of the contractile ring.

199 **The transgenerational accumulation of repressive chromatin extends lifespan via DAF-12 signaling in *C.***

elegans Scott Roques[1], Jaime Croft[2], Alex Beaudoin[1], Teresa Lee[1] [1]Biological Sciences, University of Massachusetts Lowell, [2]University of Massachusetts Lowell

Longevity is a complex phenotype that integrates genetic information with environmental cues. Recent work has shown that epigenetic components, like histone modifications, also play an important role in regulating lifespan. We discovered that *C. elegans* populations with higher levels of repressive H3K9me2 acquire longevity in a transgenerational manner: mutations in WDR-5, a component of the H3K9me-modifying COMPASS/MLL complex, or in JHDM-1, a putative H3K9me demethylase, both cause a gradual increase in lifespan over 10-20 generations. Longevity in both mutants requires germline H3K9me2 deposition, which specifically accumulates at genes expressed in the germline. In *C. elegans*, several longevity pathways convene at DAF-12, the nuclear hormone receptor involved in signaling between somatic and germline tissues. DAF-12 also acts as a key regulator of entry into the alternative L3-stage called dauer in response to environmental stress. Here, we investigate the involvement of DAF-12 in our transgenerational longevity mutants. We find that *daf-12* is necessary for both *wdr-5* and *jhdm-1* mutant populations to acquire longevity. Interestingly, a mutation in *daf-12* also suppresses the maintenance of longevity in transgenerational *wdr-5* mutant populations, but not in *jhdm-1* mutants, indicating that the lifespan extension in each mutant may occur via different downstream pathways. Finally, mutations in either *wdr-5* or *jhdm-1* partially rescued the dauer-defective phenotype of *daf-12* mutants, suggesting that chromatin landscapes may also influence entry into dauer. Taken together, these findings highlight the complex relationship between chromatin state and germline-to-soma signaling during important life history decisions.

200 **DROSHA forms distinct nuclear condensates in *C. elegans*** Bing Yang[1], Brian Galleta[2], Nasser Rusan[2], Katherine McJunkin[1] [1]National Institute of Diabetes and Digestive and Kidney Diseases, [2]National Heart, Lung and Blood Institute

Precise and specific control of miRNAs both temporally and spatially is of extreme importance, since mis-regulation of miRNAs could result in numerous abnormal phenotypes, like heart diseases and cancer. miRNA biogenesis employs a two-step mechanism. The first step is the cleavage of primary miRNA transcripts (pri-miRNA) into precursor miRNAs (pre-miRNA) by the Drosha/DGCR8 Microprocessor complex. The second step involves the Dicer nuclease, which processes the pre-miRNA into mature miRNA products. Efforts to understand the structural and biochemical properties of Microprocessor have been mostly restricted to the carboxyl domains of Drosha and DGCR8, and the function of the less conserved N-terminal regions containing the IDR (Intrinsic Disorder Region) domains in Microprocessor is poorly understood. We constructed fluorescence tagged DROSHA protein via CRISPR in *C. elegans* and observed granule-like localization in the nuclei, which suggested a phase-separated distribution of the Microprocessor. Preliminary FLIP experiments on DROSHA::GFP suggested that the granule-like structures are highly dynamic. Through co-localization analysis, we learnt that DROSHA granules are distinct from other known subnuclear organelles in *C. elegans*. To further understand the function of IDR domains in Microprocessor, we generated mutants of Microprocessor lacking the IDR domains and observed phenotypes associated with one of the IDR deletion mutants, which also showed reduction in microRNA processing activity. Taken together, we discovered that DROSHA forms distinct nuclear condensates and one of the predicted IDRs plays a critical role in mediating DROSHA activity.

201 **Mechanical force of uterine occupation enables large vesicle extrusion from proteostressed maternal neurons** Guoqiang Wang[1], Ryan Guasp[1], Sangeena Salam[1], Edward Chuang[1], Andrés Morera[1], Anna J Smart[1], David Jimenez[1], Sahana Shekhar[1], Ilija Melentijevic[1], Ken C Nguyen[2], David H Hall[2], Barth Grant[1], Monica Driscoll[1] [1]Molecular Biology and Biochemistry, Rutgers University, [2]Neuroscience, Albert Einstein College of Medicine

Large vesicle extrusion from neurons may contribute to spreading pathogenic protein aggregates and promoting inflammatory responses--two prospective mechanisms leading to neurodegenerative disease. Factors that regulate extrusion of large vesicles, such as exophers produced by proteostressed *C. elegans* touch neurons, are poorly understood. Here we document that mechanical force can significantly potentiate exopher extrusion from proteostressed neurons.

Exopher production from the *C. elegans* ALMR neuron peaks at adult day 2 or 3 under lab conditions, coinciding with the *C. elegans* reproductive peak. Genetic disruption of *C. elegans* germline, sperm, oocytes, or egg/early embryo production can strongly suppress exopher extrusion from the ALMR neurons during the peak period. Conversely, restoring egg production at the late reproductive phase through mating with males, or inducing egg retention with genetic interventions that block egg-laying, can strongly increase ALMR exopher production.

The ALMR neuron is the most active touch receptor neuron (TRN) for exopher production as compared to the other five TRNs. Interestingly ALMR is the TRN most proximal to the uterus, with the exopher-producing ALMRs mostly (~95%) located in the vicinity of the uterine egg zone. Additionally, genetically maneuvering ALM neurons away from the egg zone can strongly suppress ALMR exopher production. Moreover, over-filling the uterus with eggs can strongly promote exopher extrusion from the ALMs, AVM, PVM neurons near the egg zone, but not from the two tail-located PLM neurons. Genetic interventions

that promote ALMR exopher production are associated with expanded uterine length. In contrast, genetic interventions that suppress ALMR exopher production are associated with shorter uterine lengths. Overall TRNs close to an egg-distended uterus, show increased exopher production.

A simple hypothesis for neuronal exopher production could be that eggs produce chemical factors that stimulate exophergenesis. Our data rule out this model because filling the uterus with oocytes, dead eggs, or even injected fluid can all strongly induce ALMR exopher production. Thus, we favor a model in which the mechanical force of uterine occupation triggers exopher extrusion from the proximal proteostressed maternal neurons.

Our observations draw attention to the potential importance of mechanical signaling in extracellular vesicle production and in aggregate spreading mechanisms, and make a case for enhanced attention to mechanobiology in neurodegenerative disease.

202 **Emerging high-content methods for aging research in *C. elegans*** George L. Sutphin, Samuel Freitas, Emily A. Gardea, Vanessa Silbar Molecular & Cellular Biology, University of Arizona

This seminar will present a new robotic imaging platform for high-content data collection in the *Caenorhabditis elegans*. *C. elegans* are a long-standing model for human aging due to their short lifespan, ease of culture in the laboratory, and the availability of powerful molecule tools. The standard approach to measuring *C. elegans* lifespan involves the labor-intensive process of manually examining animals cultured in groups on petri plates to determine whether they are still alive every 1-3 days throughout their lifespan. Advancement in automated image collection, image processing, and machine learning over the past decade has led to the development of several systems that enable automated collection of *C. elegans* lifespan data, increasing throughput while limiting subjective scoring bias. In addition to being short-lived, *C. elegans* are transparent, allowing fluorescent biomarkers reporting activity in many molecular processes to be monitored. As with lifespan, standard methods for quantifying fluorescent biomarkers are labor-intensive and involve paralyzing animals and manually capturing fluorescent images. We developed an robotic imaging platform that combines automated lifespan and healthspan data collection with simultaneous collection of fluorescent imaging data across lifespan in free-crawling *C. elegans* cultured on solid media. Our system is designed for use with single-worm culture systems, enabling dynamic changes in up to three co-expressed biomarkers to be tracked in individual animals across lifespan. The specific set of biomarkers used can be tailored to the distinct goals of each experiment. Our platform can monitor ~20,000 animals in parallel (~150,000 animals per year) and is compatible with genetic (RNAi, deletion mutants, transgenics) and pharmacological interventions. The ability to capture multiple physiological—lifespan, activity, health, body size/shape—and molecular—up to three fluorescent biomarkers—over time in the same individual animals represents a shift from high-throughput lifespan screening to high-content data collection with applications across the *C. elegans* research community.

203 **Sex-specific mechanisms of temperature-induced transposon mobilization in spermatocytes** Diana E Libuda Biology, University of Oregon

Sexually-reproducing organisms generate haploid gametes, such as sperm and eggs, to transmit their genome to the next generation. All tissues are susceptible to dramatic increases in temperature, however, developing sperm are unusually sensitive to small temperature fluctuations. In contrast to oogenesis, spermatogenesis requires a narrow isotherm of 2-7°C below core body temperature to ensure fertility. One of the research focuses of my group is to elucidate the sexually dimorphic mechanisms that cause temperature-induced male infertility and genomic instability. In my talk, I will discuss our recent work with the nematode *C. elegans* and the zebrafish *Danio rerio* that has identified how small RNA pathways and meiotic chromosome structures contribute to temperature-induced transposon mobilization specifically in spermatogenesis.

204 **Modeling the fitness cost of chromosomal duplication identifies determinants of aneuploidy toxicity in budding yeast** Julie CM Rojas[1], James Hose[2], H Auguste Dutcher[2], Michael Place[2], Audrey P Gasch[2] [1]Genetics-Biotechnology Center, University of Wisconsin-Madison, [2]Genetics, University of Wisconsin - Madison

Whole-chromosome aneuploidy, characterized by an abnormal number of chromosomes, affects growth and fitness across most eukaryotes. Although generally thought of as deleterious, aneuploidy often underlies adaptation to hostile environments and is prevalent in 80% of human tumors. The reason for this discordance is not clear, in part because the cellular consequences of chromosome imbalance remain unknown. To understand the molecular determinants of aneuploidy effects, we generated a panel of wild-isolate S. cerevisiae strains in which each of the 16 chromosomes is duplicated and then used comparative mathematical modeling of the strains' fitness costs to test specific biological hypotheses. The distribution of measured fitness costs is relatively well explained by simply summing the number of genes per chromosome, explaining 62% of the variance in growth rates, consistent with the hypothesis that genetic burden affects fitness. However, this simple model does not consider the specific properties of genes amplified on those chromosomes or non-genic features. To explore the impact of specific chromosomal features, we used a low-copy plasmid library to measure the fitness effects of duplicating

genes individually and then performed lasso regression to define the determinants of aneuploidy fitness effects. We outline a multi-factorial model in which the impacts of aneuploidy are explained by the additive effect of individual-gene fitness costs along with specific classes of non-coding RNAs present on each chromosome. We confirmed the model experimentally by showing that the duplication of snoRNAs is detrimental while amplification of tRNAs improves strain growth, especially in a sensitized mutant background lacking RNA binding protein Ssd1. In all, our model explains 75-94% of the variance in aneuploid growth rates and reveals important new insights into the mechanisms of aneuploidy toxicity.

205 **SUMO modifies Hop1^{HORMAD1} at distinct sites to facilitate checkpoint signaling and inter-homolog crossing over** Sara Hariri[1], Marry Wallace Corrigan[2], Regina Bohn[2], Nikhil Bhagwat[2] [1]Microbiology and Molecular genetics, UC Davis, [2]UC Davis

Protein modification by the ubiquitin-related modifier, SUMO, helps orchestrate the complex events of meiotic prophase I, including chromosome pairing, synapsis, and recombination. Our recent proteomics analysis identified cohorts of SUMO-modified proteins involved in each step of meiotic recombination in budding yeast. One SUMO target is the conserved HORMA-domain protein, Hop1^{HORMAD1}, a dynamic component of chromosome axes that mediates interactions between homologous chromosomes. Hop1 facilitates the initiation of recombination by DNA double-strand breaks, promotes recombination between homologous chromosomes rather than sister chromatids, and mediates DNA damage checkpoint signaling by activating the effector kinase, Mek1^{CHK2}. Our recent analysis points to roles of SUMOylation in the inter-homolog bias and checkpoint signaling functions of Hop1. We show that a SUMOylation-deficient mutation (*hop1-15KR*) causes reduced levels of inter-homolog recombination (crossing over) and bypasses the meiotic prophase-I arrest phenotypes of mutants defective for recombination and chromosome synapsis. Moreover, the activity of the apical kinase Mek1 is decreased in *hop1-15KR* mutant cells, further confirming a defect in checkpoint signaling. To understand how SUMOylation facilitates Hop1 function, I will analyze the SUMO-deficient hop1-15KR protein for: (i) protein stability; (ii) phosphorylation by the DSB sensor kinases Mec1^{ATR} and Tel1^{ATM}; nuclear and chromosomal localization dynamics; and interaction with its partner proteins. Together, this analysis will provide insights into how SUMOylation regulates key aspects of meiotic prophase-I via modification of Hop1.

206 **Strategic targeting of Cas9 nickase induces large segmental duplications** Yuki Sugiyama, Satoshi Okada, Takashi Ito Kyushu University

The genesis of genomic structural variations (SVs) is critical in genome evolution. A method to induce specific SVs at the targeted genomic region is essential to understand how genomes evolve via SVs. However, such a method is yet to be established. In this study, we attempted to develop a method to induce specific segmental duplications in the genome using paired-nicking by nCas9.

We constructed a genetic reporter system to detect a segmental duplication in the genome of the budding yeast *Saccharomyces cerevisiae*. We made a strain in which chromosome IV carries two fragments derived from the *URA3* gene (*RA3* and *UR,* harboring a 391-bp overlapping sequence) at the interval of 47-kb containing replication origin. A recombinational repair event between the overlapping sequences duplicates the 47-kb segment between *RA3* and *UR* to reconstitute the *URA3* gene. The cells harboring the segmental duplication are Ura$^+$ or can grow on the medium lacking uracil. When two single-strand breaks were induced simultaneously at an upstream position of *RA3* and a downstream position of *UR* using Cas9(D10A) nickase with two guide RNAs designed at the two positions, ~15 % of surviving colonies were Ura$^+$. Long-read sequencing confirmed the tandem duplication of the 47-kb segment. We named this method “Paired-Nicking-induced Amplification (PNAmp).”

We investigated the maximum size of segments that PNAmp can duplicate. We made a series of strains harboring various intervals between *RA3* and *UR.* Surprisingly, even when the interval was ~1 Mb (covering ~90% of the right arm of chromosome IV), 10% of the surviving colonies were Ura$^+$.

To verify that PNAmp can induce SD in a natural genomic context, we targeted a 117-kb region bounded by the Ty1 retrotransposable elements, which share ~96% homology, on chromosome IV. After inducing paired nicks upstream of one Ty1 element and downstream of the other, we performed whole-genome sequencing to demonstrate not only a 1.15-fold increase in normalized read counts of the target region, but also the presence of reads covering the junction of the intended SD. These results indicated that PNAmp can use naturally occurring homologous, but not identical, sequences to induce SDs.

In addition, we showed that PNAmp using an appropriate splint sequence (termed “splinted PNAmp”) allows efficient duplication of genomic segments even when they lack flanking homologous sequences.

In summary, we established a method to induce specific large-scale segmental duplication at high efficiency using paired-nicking by nCas9.

207 **Expanded CCTG tetranucleotide DNA repeat tracts associated with Myotonic Dystrophy Type-2 elevate DNA fragility *in vivo* in a length-dependent manner** Jane C. Kim, Zaid M. Salah, Madison B. Atchley, Clarissa I. Garcia, Luis A. Hernandez California State University San Marcos

An expanded tract of CCTG tetranucleotide DNA repeats in the *CNBP* gene is the mutation responsible for the genetic disorder Myotonic Dystrophy Type 2 (DM2). Strikingly, affected individuals can have 75-11,000 CCTG repeats at the locus. The molecular mechanisms and genetic control responsible for CCTG repeat expansions and contractions is not well understood. We used a direct duplication recombination assay (DDRA) to study DNA fragility of these repeats and investigate how this fragility is affected by repeat orientation, length, and persistence of single stranded DNA. We found that 100 repeats of either CCTG or reverse complementary CAGG repeats elevate DNA fragility above a no repeat control, though there was no significant difference between the two orientations. Investigating repeat length in one orientation, we found that DNA fragility was elevated upon exceeding 60 repeats with a non-linear increase for 138 repeats. DNA fragility was elevated in strains with point mutations in *RFA1*, which encodes the largest subunit of the heterotrimeric Replication Protein A. Thus, we propose that greater persistence of single-stranded DNA elevates CAGG/CCTG fragility, and repair of these double strand breaks contributes to the massive DNA expansions and contractions observed in DM2 patients and their offspring.

208 **Determining the role of a major microtubule binding pathway in the regulation and function of kinetochore** Nairita Maitra, Devin Edwards, Sue Biggins Fred Hutch Cancer Center

A cell must ensure equal partitioning of its duplicated chromosomes each time it divides. Error in this process can result in either gain or loss of genetic material, leading to abnormalities found in cancer and birth defects. Cells utilize the force generated by the microtubule-based spindle to pull the chromosomes apart. A megadalton protein complex called kinetochore assembles at the centromere of each chromosome, recognizes these pulling forces, and eventually transmits them to the DNA, facilitating the separation of the genetic material. The tension on the kinetochore-microtubule attachments keeps changing throughout the cell cycle. During metaphase, the attachments are under high tension, as the pulling force of the microtubules is opposed by the linkage between the duplicated chromosomes. However, cells often make improper attachments that lack tension. Error correction pathway detects such attachments, destabilizes the kinetochore-microtubule binding, giving the cells another chance to build proper attachments. While these activities during the metaphase are necessary, during anaphase, the tension between the kinetochore and microtubule drops as the cohesion between the duplicated chromosome is gone, and the microtubules also start shortening. Hence, asking how the kinetochores remain attached to the microtubules during anaphase when the tension is low is important. Two competitive pathways recruit the major microtubule-binding protein Ndc80c to the kinetochore- Mis12c and Cnn1 pathway. The Cnn1 pathway gets enriched at the kinetochore during anaphase, making it a potential pathway that could stabilize the low-tension attachment during anaphase. Using budding yeast as my model organism, I aim to study how the Cnn1 pathway regulates kinetochore assembly and function during the cell cycle, especially in anaphase. Recent studies show that SUMOylation is critical in kinetochore assembly and function. Cnn1 protein has a conserved SUMO interacting motif (SIM) that can interact with another kinetochore protein via SUMOylation. Purifying Cnn1 from wild-type and $Cnn1^{SIM}$ mutant showed that the level of Stu2, a microtubule polymerase, decreases in the $Cnn1^{SIM}$ mutant, which is cell cycle-independent and unique to the Cnn1 pathway. Moreover, our biophysical assay shows that the $Cnn1^{SIM}$ mutant has weak kinetochore-microtubule attachments during anaphase compared to the wild type cells. Hence, currently, I am investigating how the interaction is happening and how it affects the kinetochore-microtubule attachments. The exclusive interaction of a microtubule polymerase like Stu2 with the Mis12c and Cnn1 pathway could demonstrate how the Cnn1 pathway might maintain kinetochore-microtubule attachments under low tension.

209 **Elevated and skewed dNTP pools alter multiple activities at the replisome** Jennifer A Surtees, Natalie Lamb, Luke Hess Biochemistry, University at Buffalo

The fidelity of DNA replication is critical in ensuring the stability of the genome from cell division to the next. Accurate DNA synthesis by replicative DNA requires appropriate levels and ratios of deoxyribonucleotide triphosphates (dNTPs), the building blocks of DNA, and is enhanced by the polymerase proofreading exonuclease function. The mismatch repair (MMR) system functions as a spell-check for DNA replication, detecting and directing repair of replication errors that evade proofreading. When dNTP pools are dysregulated, it interferes with the normal functioning of the replisome. Elevated dNTP pools lead to an increased rate of nucleotide misincorporation, increase the rate of DNA replication and alter the number of replication origins that are activated. We previously used deep sequencing to demonstrate that altering the dNTP pools in different ways (elevated, skewed) leads to distinct mutation profiles. These misincorporation events are substrates for MMR, but as the mutation rates increase, MMR can become saturated. Thus, altered dNTPs can promote mutagenesis that can lead to cancer. At the same time, cancer cells have elevated dNTP pools to maintain rapid proliferation. This can, in turn, lead to further mutagenesis and promote the molecular evolution of the cancer, providing a selective advantage.

In a previous synthetic lethal (SGA) screen in *Saccharomyces cerevisiae,* utilizing *rnr1Y285A,* which resulted in 20-fold excess dCTP and dTTP, we identified almost all of the genes involved in the mismatch repair (MMR) pathway. The contribution of the MMR system to maintaining replication fidelity is well established, but our results highlight an essential role of the pathway in the presence of skewed dNTP pools. Deleting MMR genes in combination with *rnr1Y285A* resulted in compromised cellular fitness and a defect in cell cycle progression. Our data suggest that the errors accumulated under elevated dNTP pools is sufficient to activate the DNA damage response.

We were also intrigued by the observation that altered levels/ratios of dNTP pools lead to increased frameshift slippage events and the possibility that this might affect the rate of trinucleotide repeat (TNR) expansions. TNR expansions occur in the presence of *CNG* repeat sequences in the DNA and promote neurodegenerative diseases such as Huntington's Disease or myotonic dystrophy type I. To test this, we used our yeast *in vivo* TNR reporter system to determine the rate of expansions in genetic backgrounds that alter dNTP pools. Our preliminary data indicate that in *rnr1D57N* (2-fold increase in all 4 dNTPs) resulted in decreased $(CAG)_{25}$ expansions compared to wild type. We propose that alterations at the replication fork can modify the probability of generating a slipped strand TNR structure that can be expanded.

These data are consistent with our hypothesis that elevated dNTP pools fundamentally change the activity of the replisome.

210 **Evolution in action: extreme genomic and phenotypic variation in RNAi/meiotic drive systems** Garima Setia[1], Bernard Kim[2], Holly Mudgett[3], Taiya Jarva[1], Patricia Foley[3], Dmitri Petrov[2], Colin Meiklejohn[3], Eric C. Lai[1] [1]MSKCC, [2]Stanford, [3]University of Nebraska

Selfish meiotic drive systems can distort progeny sex-ratio and/or induce sterility. Although widespread in nature, the molecular mechanisms of meiotic drive, and how they are silenced to restore Mendelian segregation, remain largely mysterious. Importantly, their rapid evolution means that classic model organisms are often not suited to reveal their fundamental features, breadth and impact. Using *Drosophila simulans* (*Dsim*), we uncovered critical roles for hpRNA-class RNAi substrates to suppress incipient sex chromosome conflicts. These arose in the *simulans*-clade ancestor, and are not found in their close relative *D. melanogaster*. Knockouts of *Dsim* hpRNA-siRNA loci (Nmy and Tmy) exhibit profound defects in the male germline, leading to loss of male progeny or outright sterility. These phenotypes are due to de-repression of recently-emerged members of the «Dox» family, which derive in part from protamine, which condenses the paternal genome. Strikingly, analysis of nanopore genomes from a panel (~20) of wild *Dsim* strains indicates that nearly all individual lines examined harbor different numbers of Dox family genes on their X chromosomes. Notably, these include many *de novo* insertions that were not present in the initial PacBio *Dsim* genome available for study. Moreover, numerous wild X chromosomes exhibit highly variable drive activities when assayed in *nmy* or *tmy* mutants, in that several X chromosomes can partially or completely suppress sex ratio or sterility defects, but some can enhance these hpRNA mutant phenotypes. We hope to gain mechanistic insights into sex ratio and sterility drive from these long-read genomes and genetic interactions. Overall, they uncover broad, unanticipated complexity in rapidly-evolving sex chromosome conflicts and may potentially foment speciation.

211 **Unveiling a novel exonuclease driving paternal mitochondrial DNA elimination in *Drosophila* spermatogenesis** Zhe Chen[1], Zong-heng Wang[2], Fan Zhang[2], Michaela Yamine[2], Christian Combs[2], Hong Xu[2] [1]NHLBI, National Institutes of Health, [2]National Institutes of Health

The exclusive maternal transmission of mitochondrial DNA (mtDNA) in animals was traditionally attributed to passive differences in cytoplasmic contents between eggs and sperm. However, recent studies have revealed active mechanisms involved in the elimination of mtDNA during spermatogenesis in various species. Despite this progress, the factors driving this process and the physiological significance of mtDNA clearance remain largely unknown. In our investigation, we identified POLDIP2 as a novel mtDNA exonuclease that plays a central role in this elimination process. POLDIP2, encoded by the CG12162 locus, exhibits specific enrichment in the late stages of spermatogenesis when mtDNA clearance occurs. Although *Poldip2* mutant flies exhibited normal spermatogenesis progression, they produced fewer mature sperms that frequently contained multiple copies of mtDNA, indicating the essential role of POLDIP2 in mtDNA clearance. Notably, we discovered that POLDIP2 is an exonuclease with a preference for degrading single-stranded DNA and double-stranded DNA with breaks. Intriguingly, ectopic expression of a mitochondrially targeted *E.coli* exonuclease III in *Poldip2* mutant flies effectively mitigated residual mtDNA and substantially restored male fertility and nuDNA fragmentation, highlighting the detrimental consequences of persisting mtDNA in mature sperm cells. To our knowledge, POLDIP2 represents the first factor identified to specifically enforce maternal inheritance of mitochondrial genomes. This discovery opens avenues for future investigations into the physiological significance and underlying mechanisms of this highly conserved yet enigmatic uniparental inheritance of mtDNA.

212 **Specialized translational machinery is required for spermatogenesis in *Drosophila melanogaster*** Brook L Falk[1,2], Yonit Tsatskis[2], Celine Salhab[2,3], Kirsten Arnold[4], Sarah C Hughes[4], Julie A Brill[1,2] [1]Department of Molecular Genetics, University of

Toronto, [2]Cell Biology Program, The Hospital for Sick Children, [3]Human Biology Program, University of Toronto, [4]Department of Medical Genetics, University of Alberta

Drosophila melanogaster sperm development relies on extensive post-transcriptional regulation as thousands of transcripts are preserved to be translated at later stages when they are required. A key step in translation initiation is binding of eukaryotic initiation factor 4E (eIF4E) to the 5' mRNA cap. *D. melanogaster* has multiple paralogues of eIF4E, including four testis-enriched paralogues (eIF4E-3, -4, -5 and -7). We and our collaborators previously discovered that eIF4E-3 and -5 are both needed for male fertility and formation of mature sperm. Their roles, however, are stage specific as eIF4E-3 first appears pre-meiotically and is required for meiotic chromosome segregation and cytokinesis whereas eIF4E-5 is required for the final stage of spermiogenesis called individualization. During individualization, organelles and cytoplasmic components not needed in mature sperm are stripped away by non-apoptotic caspase activity. In flies lacking eIF4E-5, the gradient of the caspase regulatory protein, Soti*,* is disrupted and caspase activity is dysregulated, resulting in the absence of mature sperm. The specific functions of the testis eIF4Es are likely conferred by differences in gene expression, mRNA localization and translation, or protein sequence. Therefore, we generated transgenes to define the gene-specific sequences that are required for eIF4E-3 and -5 function. We found that fertility of *eIF4E-3* mutants is rescued by expression of a 3xFLAG-tagged genomic eIF4E-3 transgene that includes its introns and upstream and downstream regulatory regions, whereas fertility of *eIF4E-5* mutants is restored by expression of 3xFLAG-tagged eIF4E-5 transgenes containing either the endogenous genomic region or a cDNA under control of a spermatocyte-specific promoter (*b2-tubulin).* We have also generated domain swap transgenes to test the requirement for the non-conserved portions of eIF4E-3 and -5. To further the mechanistic understanding of the testis eIF4Es, I will use the tagged constructs in affinity purification coupled with mass spectrometry and RNA-immunoprecipitation experiments. In addition, I generated eIF4E-5 transgenes tagged with the promiscuous biotin ligase miniTurbo to aid in the identification of transiernt protein interactors. Overall, eIF4E-3 and -5 are stage specific translational regulators required to ensure correct spatiotemporal synthesis of proteins during different stages of spermatogenesis, and our results will provide insight into how these testis eIF4Es function and are regulated during spermatogenesis.

213 **Mating induces ecdysone signaling in the *Drosophila* testis niche disrupts soma-germline contacts and stem cell cytokinesis** Tiffany V. Roach, Kari F. Lenhart Biology, Drexel University

Germline maintenance relies on adult stem cells to continually replenish lost gametes over a lifetime and respond to external cues altering the demands on the tissue. Mating worsens germline homeostasis over time, yet a negative impact on stem cell behavior has not been explored. Using extended live imaging of the *Drosophila* testis stem cell niche, we find that short periods of mating in young males disrupts cytokinesis in germline stem cells (GSCs). This defect leads to failure of abscission, preventing release of differentiating cells from the niche. We find that GSC abscission failure is caused by increased ecdysone hormone signaling induced upon mating, which leads to disrupted somatic encystment of the germline. Abscission failure is rescued by isolating males from females but recurs with resumption of mating. Importantly, reiterative mating also leads to increased GSC loss, requiring increased restoration of stem cells via symmetric renewal and de-differentiation. Together, these results suggest a model whereby acute mating results in hormonal changes that negatively impact GSC cytokinesis but preserves the stem cell population. Future work will focus on directly visualizing somatic stem cells and their immediate daughters to observe encystment behaviors in real time under homeostatic as well as stressful conditions.

214 **Close encounters with Oskar: proteomic profiling of germ granule interactions and beyond** Kwan Yin Lee[1], Harathi Jonnagaddala[2], Marian Kalocsay[2], Elizabeth R Gavis[1] [1]Molecular Biology, Princeton University, [2]Experimental Radiation Oncology, The University of Texas MD Anderson Cancer Center

A conserved feature of germline development in animals is the formation of cytoplasmic ribonucleoprotein bodies called germ granules. Germ granules are phase transitioned condensates comprising a protein scaffold into which numerous mRNAs encoding proteins required for germline specification, proliferation, and maintenance are incorporated. In *Drosophila melanogaster,* recent work has shown that germ granules are remodeled during primordial germ cell (PGC) development so that they can be repurposed for distinct functions. Remodeling occurs by the recruitment of protein factors, perhaps leveraging the properties of germ granules as phase transitioned entities that can exchange protein content with the environment over time. Is this a general strategy to generate different outcomes in the post-transcriptional regulation of mRNAs within germ granules? A dedicated mechanism is necessary because a germ granule can contain up to hundreds of different mRNAs. Differences in the regulation of several germ granule mRNAs have been known for over a decade, but mechanisms underlying these observations remain unknown. Oskar is a core germ granule protein in *Drosophila melanogaster* that is present throughout the lifecycle of the germ granule. Granule assembly is driven by Oskar which nucleates the recruitment of other germ granule scaffold proteins through both protein-protein and multivalent interactions. As part of the germ granule, Oskar also binds and post-transcriptionally regulates specific mRNAs. To expand our understanding of how germ granules contribute to post-transcriptional regulation, we used a proximity dependent biotinylation approach to capture the protein milieu that

Oskar is immersed in from oogenesis to embryogenesis. Oskar was conjugated to TurboID, an engineered biotin ligase that catalyzes promiscuous biotinylation of proteins within a radius of several nanometers. Using multiplexed TMT quantitative mass spectrometry, we generated Oskar-TurboID datasets in ovary samples and in samples at multiple timepoints during embryogenesis. Our experiments reveal dramatic changes in the proteins biotinylated by Oskar-TurboID in embryogenesis compared to oogenesis. Oskar-TurboID biotinylates hundreds of additional factors at the onset of embryogenesis suggesting widespread remodeling of the germ granules and their environment upon fertilization. We hypothesize that these dramatic changes represent a developmental program driving post-transcriptional regulation of germ granule mRNAs to prepare the *Drosophila* embryo for PGC formation. Live imaging and antibody staining confirmed the dynamic localization of protein candidates from our Oskar-TurboID time course. Therefore, our Oskar-TurboID approach comprehensively records molecular encounters of Oskar *in vivo*.

215 **Drosophila H2A.Z regulates developmental timing and the global transcriptome** Pakinee Phromsiri, Noah Reger, Fanju Meng, Claire Makowski, Patrick Murphy, Michael Welte University of Rochester

In early Drosophila embryos, the variant histone H2Av is necessary for the upregulation of numerous genes during zygotic gene activation (ZGA) and is already present at many transcription start sites before ZGA. We discovered that nuclear H2Av levels increase gradually with each nuclear cycle (NC), from NC9 to NC14. To determine whether this stepwise increase is functionally important, we compared embryos from mothers with one (*1X H2Av*), two (*2X H2Av*; wild type) and four (*4X H2Av*) copies of the *H2Av* gene. Even though these three genotypes develop grossly normal, they display consistent differences in the length of NC13, compared to wild type. NC13 is slowed down in *1X H2Av* and accelerated in *4X H2Av* embryos. We also observed a similar acceleration of NC13 in *Jabba* mutant embryos in which H2Av is not properly sequestered in the cytoplasm and over accumulates in nuclei (similar to *4X H2Av*). These observations suggest that the nuclear levels of H2Av control the timing of certain developmental events around ZGA. H2Av is the sole H2A variant in Drosophila, combining H2A.X and H2A.Z functions. H2A.X is phosphorylated upon DNA damage and promotes DNA repair; H2A.Z functions in transcription and chromatin structure. Using transgenes encoding a mutant H2Av unable to be phosphorylated, we found that H2Av's H2A.X function is dispensable for the NC13 speed up. Since H2A.Z is involved in transcriptional regulation, we compared wild type and *4X H2Av* embryos by RNA-seq. In NC13 and early NC14, we found significant differences in abundance for hundreds of transcripts. These differences appear during zygote development as the global transcriptome of freshly laid embryos was largely identical between the two genotypes. At the later timepoints, most of the changes reflect premature degradation of maternally provided transcripts; in NC13 *4x H2Av* embryos, these messages are already reduced to levels that in the wild type are reached only in NC14. We also find that a small fraction of zygotic transcripts (46 out of 6231 detected) display altered expression levels, possibly reflecting premature or delayed activation of their transcription. We are currently testing whether such changes to transcriptional regulation drive NC13 acceleration and premature degradation of maternal messages.

216 **Cuticle nanopatterning in the *Drosophila* olfactory organs is controlled by the ER-resident *Osiris* family protein Gore-tex.** Shigeo Hayashi, Sachi Inagaki, Takeshi Itabashi, Atsuko Iwane RIKEN Center for Biosystems Dynamic Research

Insect cuticle structures are extensively modified to meet various physiological demands, such as decorating the surface with structural color and superhydrophobicity in the ocular lens. The biological mechanism of insect cuticle nanopatterning is totally unknown. Small pores of ~30 nm diameter in the cuticle of olfactory organs permit access of chemical signals to sensory neurons inside the cuticle. We previously reported that nanopore formation and olfactory response fail in the mutant of the *gore-tex (gox)/Osiris23 (Osi23)* gene (Ando et al., 2019). *Osi* family genes are expressed in various cuticle-secreting cells and encode endosome-associated transmembrane proteins. Their sequences are highly conserved in insects but absent in the genome of other arthropods, suggesting that *Osi* genes are involved in insect-specific cuticle morphogenesis. To understand the role of the *gox/Osi23* gene, we performed ultrastructural analyses of olfactory hair cells in the early stage of cuticle formation at 42 to 52 hours after puparium formation. Reconstruction of membrane structures from 3D stacked images of the focused ion beam SEM revealed interconnected networks of endoplasmic reticulum spanning the entire length of olfactory hair cells. Gox-labeled endosomes were found to span the position connecting the ER network to the cell surface. We show that Gox restricts subcortical ER by promoting ER phagy, which is essential for nanopore formation. We will present the identification of the molecule linking Gox to ER-phagy and the downstream cellular events linking the membrane dynamics to the nano-level cuticle assembly.

217 **Combinatorial role of signaling factors in the maintenance of Drosophila germline stem cell fate** Amelie Raz, Hafidh Hassan, Yukiko Yamashita Whitehead Institute for Biomedical Research

In the Drosophila testis, GSCs divide asymmetrically to simultaneously self-renew and generate differentiating spermatogonia which, in turn, will undergo multiple symmetric divisions before entering meiosis. Such divergent cell behaviors – asymmetric division and self-renewal by GSCs vs. symmetric division and differentiation by their daughters – suggests that the GSC-

to-spermatogonia transition is a strict step-wise cell identity shift. However, contrary to this model, spermatogonia can spontaneously **de**differentiate back into GSCs; this behavior is required to reestablish GSCs after loss (e.g., after starvation). We determine that GSCs endow their daughter cells with most-to-all of the GSC transcriptome, maintaining spermatogonia in a protected, uncommitted, and dedifferentiation-competent state. However, spermatogonial dedifferentiation is typically suppressed by a lack *active* GSC-like transcription requiring reception of niche signals. We find that after GSC loss, longer-range niche factors can diffuse to spermatogonia, activating a subset of the GSC transcriptional program and triggering dedifferentiation. Upon engraftment into the niche, short-range signaling factors activate transcription of a separate set of GSC targets, returning these cells to normal GSC behavior, including self-renewing divisions. Such combinatorial behavior of two separate signaling ligands, in which reception of one, neither, or both each yields a unique and required cellular behavior (dedifferentiation, differentiation, or self-renewal, respectively) reveals for the first time why more than one ligand is required to maintain germline stem cell homeostasis. Together, this model identifies a gene regulatory path for programming germline stem cell identity during both self-renewal and dedifferentiation.

218 **Investigating the generation of asymmetric cell migration during heart development** Vanessa Gonzalez, Rebecca Burdine Molecular Biology, Princeton University

Asymmetric collective cell migration of cardiac progenitor cells (CPCs) is necessary for proper asymmetric development, left-sided placement, and function of the vertebrate heart. Errors in heart development can result in congenital heart defects, the most common structural birth defect in the United States. Patterning of the vertebrate embryo along the left-right (LR) axis is required for proper positioning and asymmetric development of the heart. The first left-right asymmetry in zebrafish heart development is produced by “jogging”: the asymmetric migration of CPCs to produce a left-lateralized heart tube. We and others have shown that the jogging of CPCs is required for proper LR positioning of the heart and dependent on left-sided Nodal signaling. Asymmetric Nodal signaling results in transcription factor FoxH1-mediated gene expression changes that produce faster CPC migration rates in left-sided CPCs, suggesting that transcriptional changes are critical for establishing the heart’s asymmetry. To identify how Nodal influences gene expression to increase left-sided CPC migration rates, I utilized single-cell RNA sequencing to characterize asymmetric transcriptional events during CPC migration. With this data, I have identified a set of genes that are differentially expressed between left-sided and right-sided CPCs; these candidate genes are all components or regulators of the cytoskeleton and potential novel Nodal signaling targets that are important for proper LR asymmetry of the heart. More specifically, I find that several actin genes and F-actin regulator genes are upregulated in left-sided CPCs. We then find that the F-actin cytoskeleton itself is asymmetrically present and increased in left-sided CPCs in response to Nodal signaling; loss of Nodal signaling in *southpaw* mutants results in symmetric F-actin. Additionally, I am currently characterizing a promising candidate gene, *bves*, to determine its function in jogging. *bves* is known to promote tight junction (TJ) formation, which enables faster collective cell migration. I hypothesize that *bves* upregulation by Nodal increases TJ formation and function in left-sided CPCs, and that this is critical for maintaining epithelial integrity and cell migration rates during jogging. *bves* mutants demonstrate LR cardiac laterality issues and immunofluorescence demonstrates that *bves* and *ZO-1*, a canonical TJ protein, co-localize in the jogging heart, suggesting a role for *bves* and TJs in LR cardiac asymmetry. Ongoing experiments will continue to characterize the genes and cytoskeletal components necessary for asymmetric CPC migration and proper jogging to establish asymmetry in the vertebrate heart. My work is advancing our understanding of the genetic and cellular basis of asymmetric heart development, thus shedding light on the processes that may be affected in congenital heart defects.

219 **Dissecting the cell type-specific roles of Hand2 during zebrafish cardiac development** Yanli Xu[1], Rupal Gehlot[1], Samuel Capon[1], Marga Albu[1], Jonas Gretz[1], Joshua Bloomekatz[2], Kenny Mattonet[1], Dubravka Vucicevic[3], Sweta Talyan[4], Stefan Günther[4], Mario Looso[5], Beth Firulli[6], Anthony Firulli[6], Scott Lacadie[3], Deborah Yelon[7], Didier Stainier[1] [1]Developmental Genetics (Dept. III), Max Planck Institute for Heart and Lung Research, [2]The University of Mississippi University, [3]Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin Institute for Medical Systems Biology (BIMSB), [4]Max Planck Institute for Heart and Lung Research, [5]Bioinformatics Core Unit (BCU), Max Planck Institute for Heart and Lung Research, [6]Indiana Medical School, [7]University of California

The bHLH transcription factor Hand2 plays multiple roles during vertebrate organogenesis including cardiogenesis. Cardiogenesis requires the integration of diverse cell types including the myocardial and endocardial cells that form the initial layers of the heart; it also requires the orchestration of several morphogenetic processes, including trabeculation and cardiac valve formation. Despite the importance of Hand2 during cardiogenesis, much remains to be uncovered about its mechanism of action. Here we utilized advanced genetic and imaging tools in zebrafish and first observed that *hand2* is expressed in the anterior lateral plate mesoderm which gives rise to the myocardium and endocardium from the heart tube stage to the valvulogenesis and trabeculation stages. We generated several *hand2* mutant alleles and found that dimerization-deficient mutants display the null phenotype (cardia bifida) while DNA binding-deficient mutants do not. Mechanistically, we found that *Pdgfra* is a conserved Hand2 target and effector during early cardiac development. Due to the early embryonic

lethality of *hand2*-null zebrafish, we generated a floxed allele to further investigate its function in specific lineages. Knocking out *hand2* in *myl7* expressing cells (*i.e.,* cardiomyocytes) led to a late phenotype whereby the mature trabecular ridges mostly fail to form, which is correlated with a decrease in Notch signaling in the myocardial cells. Interestingly, knocking out *hand2* in *kdrl* expressing cells (*i.e.,* endothelial cells) resulted in a loss of valve interstitial cell and a hypo-trabeculated ventricle. Altogether, these results further our understanding of Hand2 function across time and space during cardiogenesis.

220 **The role of neural crest-derived cardiomyocytes in zebrafish heart development and regeneration** Julia Whittle[1], Chelsea Herdman[2], Sarah Abdul-Wajid[2], Leonard Almero[2], H. Joseph Yost[2] [1]Molecular Medicine, University of Utah, [2]University of Utah

The neural crest (NC) is a highly migratory embryonic stem cell population unique to vertebrates. It forms at the dorsal side of the neural tube, subsequently undergoing an epithelial-mesenchymal transition to migrate throughout the embryo and differentiate into a diverse population of cell types. A subpopulation of the NC migrates to the heart and differentiates into functional cardiomyocytes (NC-Cms). In zebrafish, NC-Cms make up about 12% of cardiac cells in the embryonic ventricle and are virtually indistinguishable from other cardiomyocytes in adulthood. However, NC-Cms play important roles both in zebrafish heart development and in regeneration. In development, these cells express the Notch ligand *jag2b*, postulated to guide heart trabeculation through Notch signaling. Ablation of NC-Cms during development causes mild trabeculation defects in embryos as well as adult-onset hypertrophic cardiomyopathy and reduced heart function. We observe a phenocopy of adult hypertrophic cardiomyopathy in *jag2b* knockout mutants despite successful migration of NC-Cms to the heart, suggesting that *jag2b*-driven Notch signaling from NC-Cms is also necessary for adult heart function. In addition to their role in development, NC-Cms appear to activate during injury repair of the adult heart. The NC transcription factor *sox10* is expressed in zebrafish hearts 7 days post injury, enriched in proliferating cells. In contrast, NC-Cm ablated fish display a noticeable reduction in heart regeneration markers such as *nppb* and *raldh2*, and lack *sox10* expression at the site of injury. It appears that NC-Cms may revert to an embryonic NC-like state during heart regeneration to proliferate and rebuild missing tissue. We are also currently investigating the expression of *jag2b* at the site of injury, to determine if it reprises its role in heart development during regeneration. The neural crest guides development by differentiating into a broad array of unique cell types throughout the animal- however it appears that this NC-Cm population, after differentiation into functional cardiomyocytes, maintains some degree of elasticity and specialization well into adulthood, perhaps by maintaining a distinct epigenetic memory and the ability to re-activate embryonic capabilities of cell signaling and tissue building. To address this, we are currently exploring the chromatin structure of these cells, as well as their expression profiles and behaviors during regeneration.

221 **A regulatory network of Sox and Six transcription factors initiate a cell fate transformation during hearing regeneration in adult zebrafish** Erin Jimenez[1], Claire C Slevin[2], Wei Song[3], Zelin Chen[4], Stephen Frederickson[2], Derek Gildea[2], Weiwei Wu[2], Abdel G Elkahloun[2], Ivan Ovcharenko[3], Shawn M Burgess[2] [1]Biology, Johns Hopkins University, [2]National Human Genome Research Institute, [3]NCBI, [4]Southern Marine Science and Engineering Guangdong Laboratory

Using adult zebrafish inner ears as a model for sensorineural regeneration, we performed a targeted ablation of the mechanosensory receptors in the utricle and saccule and characterized the single-cell epigenome and transcriptome at consecutive time-points following hair cell ablation. Using deep learning on the regeneration-induced open chromatin sequences, we were able to identify unique, cell-specific transcription factor (TF) motif patterns enriched in the raw data. We correlated enhancer activity with gene expression to identify gene regulatory networks. A clear pattern of overlapping Sox- and Six-family transcription factor gene expression and binding motifs was detected, suggesting a combinatorial program of TFs driving regeneration and cell identity. Pseudo-time analysis of single-cell transcriptomic data demonstrated that the support cells within the sensory epithelium changed cell identity to a more pluripotent “progenitor” cell population that could either differentiate into hair cells or return to a support cell identity. We showed that sox2 becomes enriched in the progenitor cells and is reduced again when the cells differentiate in either direction. Analysis of the scATAC-seq data identified a 2.6 kb DNA sequence element upstream of the sox2 promoter that dynamically changed in accessibility during hair cell regeneration. When deleted, the upstream regulator of sox2 showed a dominant phenotype that resulted in a hair cell regeneration specific deficit in both the lateral line and adult inner ear.

222 **Neutrophils facilitate the epicardial regenerative response after heart injury** Elizabeth Peterson, Jisheng Sun, Xin Chen, Jinhu Wang School of Medicine, Emory University

Title: Neutrophils facilitate the epicardial regenerative response after heart injury

Introduction: As neutrophils are the first responders to tissue damage and effective heart restoration requires the systematic control of inflammation induction and resolution, we speculated that neutrophils play a critical role in the transition to heart

regeneration in zebrafish.

Methods: We utilized reporters and immunofluorescence staining to visualize neutrophils, cardiac cells, and activation markers after cardiac injury. To limit neutrophils, we incubated fish with the drug AT7519 from 4-6 hours post-amputation (hpa). For scRNA-seq of 1 day post-amputation (dpa) cardiac neutrophils, we isolated $EGFP^+$ cells by FACS from amputated *lyz:*EGFP fish hearts for 10x Genomics' scRNA-seq. We used SU5402 from 27-32 hpa to limit FGF signaling.

Results: Neutrophils mobilized to the injury site, peaking at 1 dpa and resolving by 3 dpa. Remarkably, these 1 dpa neutrophils localized near activated/proliferating epicardial cells in the wound. To further analyze this cell-cell interaction, we employed drug treatment to limit neutrophil infiltration and detected impaired cardiac regeneration. Neutrophil depletion also significantly reduced epicardial cell expansion, proliferation, and activation. scRNA-seq of 1 dpa neutrophils identified a cell cluster enriched with cell proliferation signaling pathways, including the FGF, MAPK, and ERK signaling pathways. Pharmacological inhibition of FGF indicated a requirement for FGF activity for efficient epicardial cell proliferation. Further, neutrophil depletion limited ERK signaling activation, suggesting a supportive role for neutrophils in the activation of epicardial cell proliferation.

Conclusion: Altogether, our studies revealed that neutrophils facilitate the expansion of the epicardium at the injury site to support heart regeneration.

223 **ptx3a+ fibroblast/epicardial cells provide a transient macrophage niche to promote heart regeneration** Jisheng Sun, Elizabeth Anne Peterson, Xin Chen, Jinhu Wang Emory University

Macrophages conduct critical roles in heart repair, but the cardiac tissue niche required to nurture and anchor them is poorly studied. Here, we investigated macrophage niche components in the regenerating heart. First, we analyzed cell-cell interactions through scRNA-seq datasets derived from damaged zebrafish and neonatal murine hearts. We identified a strong interaction between fibroblast/epicardial (Fb/Epi) cells and macrophages, in which outgoing signals were predominantly sent by Fb/Epi cells and incoming signals were mainly received by macrophages. Then, we visualized the association of macrophages with Fb/Epi cells and the blockage of the macrophage response after depleting Fb/Epi cells in the regenerating zebrafish heart. Moreover, we found that Fb/Epi cells with pentraxin 3 long a (ptx3a) expression displayed immune regulatory functions and ptx3a+ cell-associated macrophages exhibited M2 characteristics. Experimentally depleting ptx3a+ cells resulted in lower M2 macrophage numbers and enhanced scar formation in the wound. Further, we identified expression of multiple signaling pathways in ptx3a+ cells, including CSF pathway components, and determined that pharmacological inhibition of the csf1a pathway or csf1a knockout blocked the M2 macrophage response. Moreover, we found that genetic overexpression of csf1a enhanced the M2 macrophage response with or without heart injury, and reduced collagen in the regenerating area. Altogether, our studies illuminate a new cardiac Fb/Epi niche which mediates a beneficial macrophage response after heart injury, a process facilitated by upregulating the csf1a gene.

224 **The role of cilia genes in the development, survival and regeneration of hair cells** Hope Boldizar[1], Amanda Friedman[1], Jennifer Galdieri[1], Tess Stanley[1], María Padilla[2], Arielle Sclar[1], Tamara Stawicki[1] [1]Neuroscience, Lafayette College, [2]Biology, Lafayette College

Primary cilia play key roles in the proliferation, survival and signaling of cells throughout our body. Sensory hair cells, the cells we use for hearing and balance, have a single primary cilium known as the kinocilium. We wished to further investigate the role of the kinocilium in hair cell development and regeneration using the zebrafish lateral line. The lateral line contains a number of neuromasts which consist of both supporting cells, some of which will proliferate and develop into hair cells, and the hair cells themselves. Mutations in cilia intraflagellar transport (IFT) genes cause a loss of the kinocilium and have previously been shown to cause decreased numbers of hair cells in both the inner ear and lateral line of zebrafish. We focused specifically on mutations in the genes *ift88* and *dync2h1* which should impact both anterograde and retrograde IFT respectively. We found that similar to what had previously been shown in the inner ear of *ift88* mutants there were a small number of cells undergoing apoptosis in the lateral line of both IFT gene mutants. *ift88* mutants additionally show a reduction in mitochondria activity. In *atp6v1f* mutants which also have these reductions it has been shown that treatment with Ru360, a mitochondria calcium uniporter inhibitor, can rescue hair cell number losses. However, we failed to see this in either IFT gene mutant. Additionally, we found that cell proliferation was normal in neuromasts of these mutants during hair cell development. This suggests that cilia may be more important for the survival than the initial development of hair cells in the zebrafish lateral line. Though we cannot rule out a role for cilia in hair cell development after proliferation has happened. However, unlike in development, preliminary data suggests cilia gene mutants may have alterations in cell proliferation during hair cell regeneration. We are in the process of further analyzing hair cell regeneration in these mutants.

225 **Genetic analysis of CNS autoimmunity using the diversity of the Collaborative Cross reveals unique phenotypes**

and mechanisms Emily A Holt[1], Anna Tyler[2], Taylor Lakusta-Wong[3,4], Karolyn G Lahue[1], Katherine Hanks[1], Cory Teuscher[5], Martin T Ferris[6], J. Matthew Mahoney[2,3], Dimitry N. Krementsov[1] [1]Department of Biomedical Health Sciences, University of Vermont, [2]The Jackson Laboratory, [3]Department of Neurological Sciences, University of Vermont Larner College of Medicine, [4]Department of Neurology, University of Vermont Medical Center, [5]Department of Medicine, University of Vermont Larner College of Medicine, [6]Department of Genetics, University of North Carolina

Multiple Sclerosis (MS) is a complex disease with significant heterogeneity in disease course and progression. Genome-wide association studies (GWAS) have identified 232 loci associated with MS. However, these loci are only associated with MS incidence and not disease course, the genetic basis of which remains obscure. Here we leveraged the Collaborative Cross (CC) - a genetically diverse mouse strain panel - and myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) induced experimental autoimmune encephalomyelitis (EAE), to model genetics of MS disease course. Thirty-three CC strains were selected based on compatible MHC haplotypes ($H2^b$ and $H2^{g7}$) and EAE was monitored for 50 days. CC strains captured a wide spectrum of distinct EAE phenotypes, compared with typical chronic EAE in C57BL/6 mice, ranging from highly resistant (CC011/Unc) to severely progressive (CC028/GeniUnc). Strains also showed wide variation in disease trajectory, including monophasic disease (CC068/TauUnc and others) and relapsing remitting EAE, with ≥60% incidence in 3 strains, including CC002/Unc. In addition to classical EAE symptoms (ascending paralysis), we identified CC004/TauUnc as having a high incidence (~79%) of axial rotary (AR)-EAE, manifesting as profound ataxia and axial rotation. Sex differences in EAE course, were observed in 4 strains, including CC042/GeniUnc and CC018/Unc. Quantitative trait locus (QTL) analysis revealed distinct linkage patterns for EAE incidence and severity, with peaks on Chromosome (Chr) 4 and 14 respectively, passing genome wide significance. Machine learning-based functional candidate gene prioritization using gene network data and MS-GWAS genes as training input, ranked top candidate genes for QTL Chr4 (*Tox, Sdcbp, Chd7*) and Chr14 (*Abcc4, Gpc5, Gpc6*). Profiling of CNS infiltrating cells and histology revealed distinct pathology associated with EAE profiles, including cerebellar lesions with neutrophil infiltration in AR-EAE (CC004/TauUnc) and diffuse spinal cord lesions with reduced lymphocyte infiltration in progressive EAE (CC028/GeniUnc). Taken together, this work has led to the development of an easily implementable EAE model in CC strains, thereby expanding the EAE mouse model phenotypic repertoire and has demonstrated that high-resolution mapping can be achieved using the selected 33 CC strains. Importantly, our findings strongly support the hypothesis that heterogeneity in MS disease course is driven by natural genetic variation.

226 **A cross-species approach using an *in vivo* evaluation platform in mice demonstrates that sequence variation in human *RABEP2* modulates ischemic stroke outcomes** Han Kyu Lee, Douglas A Marchuk Molecular Genetics and Microbiology, Duke University School of Medicine

Ischemic stroke, caused by vessel blockage, results in cerebral infarction; the death of brain tissue. Previously, quantitative trait locus mapping (QTL) of cerebral infarct volume and collateral vessel number identified a single, strong genetic locus regulating both phenotypes. Additional studies identified the causative gene, encoding rabaptin, RAB GTPase binding effector protein 2 (*Rabep2*). However, there is yet no evidence that variation in the human ortholog of this gene plays any role in ischemic stroke outcomes. We established an *in vivo* evaluation platform in mice using adeno-associated virus (AAV) gene replacement and verified that both mouse and human RABEP2 rescue the mouse *Rabep2* KO ischemic stroke volume and collateral vessel phenotypes. Importantly, this cross-species complementation enabled us to experimentally investigate the functional effects of coding sequence variation in the human *RABEP2* gene. We chose four coding variants from the human population that are predicted by multiple *in silico* algorithms to be damaging to RABEP2 function. *In vitro* and *in vivo* analyses verify that all four led to decreased collateral vessel connections and increased infarct volume. Thus, there are naturally occurring loss-of-function alleles. This cross-species approach will expand the number of targets for therapeutics development for ischemic stroke.

227 **Mapping cell death and expression QTL using a barcoded library of genetically diverse macrophages** Gavin F Fujimori[1], Michael Kiritsy[2], Megan Proulx[1], Audrey Lord[3], Colton Linnertz[4], Martin Ferris[4], Clare Smith[5], Richard Baker[1], Christopher Sassetti[1] [1]Microbiology and Physiological Systems, UMass Chan Medical School, [2]Department of Medicine, Duke, [3]Boston University, [4]Genetics, UNC School of Medicine, [5]Molecular Genetics and Microbiology, Duke

Macrophages play an important role in maintaining tissue homeostasis and regulating immune responses. As innate immune cells, macrophages integrate an array of signals from cytokine, chemokine, and pattern recognition receptors. This complex network of molecular signals informs macrophage responses in any given situation. Furthermore, immune genes are under continuous adaptation due to the constant pressure of evolving pathogens. We developed a resource to take advantage of the resulting genetic diversity to study macrophages and innate immune processes. We produced a pool of DNA barcoded bone marrow progenitors derived from 59 Collaborative Cross strains. These cells can be differentiated into macrophages to study various aspects of macrophage biology. We have performed two proof-of-concept screens to confirm that we can map QTL using selection by either surface marker expression or sensitivity to a cell death inducer as phenotypes. The change in

relative abundance of each strain in the pool after selection is used to map for QTL. When mapping on Anthrax Lethal Factor (LF) induced cell death we identified a striking peak on Chromosome 11 at the well described *Nlrp1b* locus. The allele effects for susceptibility to LF induced cell death at the *Nlrp1b* locus matched those described in the literature. In addition, we identified a significant peak on chromosome 9. The allele effects at this locus explained the variance observed in strains that harbored a susceptible *Nlrp1b* allele. We performed single cell sequencing on the pool stimulated with IFNγ, Pam3CSK4, both IFNγ and Pam3CSK4, or unstimulated. Using normalized counts as a metric we were able to map several significant cis-eQTL. These include one underneath the chromosome 9 LF toxicity-associated peak that corresponds to the *Pkm* gene and matches the *Nlrp1b* allele effects. Thus, in a proof-of-concept cell death screen we identified both the expected and an unexpected peak reaching genome-wide significance. Additionally, we were able to leverage single cell sequencing to identify a cis-eQTL that we hypothesize to modify LF-mediated cell death. These results demonstrate the power of this new tool in leveraging Collaborative Cross strains to rapidly decipher the genetic basis of macrophage phenotypes in a pooled system.

228 **Genetics of Susceptibility to Mouse Myeloma leads to Co-targeting of Oncogenes and Tumor Suppressors** Beverly A Mock Lab of Cancer Biology & Genetics, CCR, NCI, NIH

Multiple Myeloma (MM) is a clonal proliferation of neoplastic plasma cells in the bone marrow. Despite recent therapeutic advances, drug resistance and MM progression is common. Mouse plasma cell tumors model certain aspects of these antibody producing neoplasms. Long-term genetic studies utilizing backcross, and congenic strain analyses coupled with positional cloning strategies and functional studies identified *Cdkn2a* (p16)*,* and *Mtor* as plasmacytoma susceptibility genes. Tumor incidence data in congenic strains carrying resistance alleles of *Cdkn2a* and *Mtor* led us to *hypothesize* that drug combinations affecting these pathways are likely to have an additive, if not synergistic effect in inhibiting tumor cell growth. When drug combinations targeting these pathways were evaluated, MYC protein degradation was found to be a common mechanism of action. Following these studies, high throughput screening of 1900 small molecule compounds identified several drug combinations that could simultaneously inhibit oncogenic MYC expression and simultaneously increase expression of the tumor suppressor, p16. Candidate combinations were evaluated for cooperative reductions in MYC protein expression in MM cells treated at IC50 doses of each drug. Cooperative reductions in viability were observed with top combinations in proteasome inhibitor-resistant and sensitive MM cell lines but did not limit normal fibroblast viability. The combinations cooperatively increased p16 and RB activity, while also enhancing cleaved caspase 3, leading to increased apoptosis. Combination-associated survival was evaluated in a transplantable Ras-driven allograft model of advanced MM which closely recapitulates myeloma in humans. Several combinations significantly prolonged survival in sublethally-irradiated C57BL/6 mice injected intracardiac with donor MM cells compared to control mice. Furthermore, and most importantly, the combination prolonged survival compared to single agents and control in three mouse models. These data identify potentially useful drug combinations for preclinical evaluation in drug-resistant MM and may ultimately reveal novel mechanisms of combined drug sensitivity.

229 **Generative adversarial networks, transfer learning, and interpretability for evolutionary inference** Sara Mathieson Computer Science, Haverford College

The broad field of generative AI has captured a worldwide audience with novel text, image, audio, and video. In evolutionary biology we have used synthetic data for decades, but created using custom simulations from evolutionary models. However, it is often challenging to create realistic simulations for populations with unique histories, which compromises the results of downstream analyses. Recently, new methods inspired by the generative AI literature have emerged as ways to automatically adapt simulations to mirror the real genomic data of any population or species.

Here I will focus specifically on a generative adversarial network (GAN) method for inferring demographic and natural selection parameters. Our method, called pg-gan, works by training a parametric generator and CNN (convolutional neural network) discriminator in concert, until there is a close match between real and simulated data. The main training algorithm requires only fast neutral simulations, which allows us to fit a demographic model. Then, we save the resulting discriminator and fine-tuned it with minimal simulations of selection, which are often computationally expensive. The final CNN allows us to predict selected regions for held aside genomic data. We demonstrate the effectiveness of our approach in simulation and with human and mosquito populations. Additionally, we show how to interpret the trained networks by clustering discriminator features based on their correlation with known summary statistics. Discriminator features include the convolutional filters themselves, the last hidden layer (where high-level information is distilled), and the final network output. Overall pg-gan has proven useful in evaluating and strengthening simulated data, especially for understudied populations that deviate from broad geographic groups.

230 **Population genetics meets ecology: a guide to simulations in continuous geography** Elizabeth T Chevy[1], Jiseon Min[2], Victoria Caudill[2], Samual Champer[3], Benjamin C Haller[3], Andrew D Kern[2], Sohini Ramachandran[1,4], Clara Rehmann[2], Chris C R

Smith[2], Silas Tittes[2], Peter L Ralph[2,5] [1]Center for Computational Molecular Biology, Brown University, [2]Institute of Ecology and Evolution, University of Oregon, [3]Department of Computational Biology, Cornell University, [4]Ecology, Evolution, and Organismal Biology, Brown University, [5]Department of Mathematics, University of Oregon

Nearly all populations we study exist in geographic space, yet demographic models, and their useful population genetic expectations, rarely accommodate continuous space. Interactions between individuals—and therefore genetic relatedness—are constrained by physical space and geographic context, and the most flexible, realistic model must capture this. However, continuous spatial modeling has its quirks. Without careful attention to the consequences of modeling choices, it can be difficult to even simulate a stable population. Population size, for example, cannot simply be "set": it is an emergent property of the simulation. Implementing complexities such as competition, sex, or environmental heterogeneity can have unintended consequences that render the simulation unrealistic or no longer useful for the modeling task.

Here, we describe a robust framework for continuous-space demographic simulations. We present some fundamental relationships, such as how genetic distance changes with geographic distance depending on how individuals migrate during their lifetimes. We also show that subtle modeling choices can have dramatic effects on properties of the simulation. For example, two models of natural selection achieve very different equilibria when selection acts on fecundity vs. mortality, and the speed at which they obtain equilibrium depends on the spatial scale.

In addition to our results, we provide practical material for researchers designing their own spatial simulations. Template code demonstrates how properties such as population density, sex, or selection can be implemented in the simulation engine SLiM. Finally, we demonstrate how to use our framework to model real systems. We provide four vignettes that explore how climate change affects geographic range, how precipitation affects an invasive species, how biogeography determines larval vs. adult abundance, and how patchy resources can drive competition.

231 **A genealogy-based framework to estimate population structure and demographic history** Charleston Chiang[1], Caoqi Fan[2] [1]Population and Public Health Sciences, University of Southern California, [2]Vibrant Therapeutics

Learning the demographic histories of nonequilibrium populations helps us understand the causes of population structure, the pattern of genetic variation, and the evolution of traits. Many existing methods to infer population structure or demographic history from genetic data use relatively low-dimensional summaries, such as the allele frequency spectra, which often ignore the linkage information between markers. In principle, much more information is available from the sequence of gene-genealogical trees, known as the ancestral recombination graph (ARG), that describes the history of sampled alleles. As a step toward capturing all the available genomic information, we introduce two methods that leverage the ARG to infer population structure and demographic histories. First, we describe a framework to infer the expected relatedness between pairs of individuals given an ARG of the sample, which we call the eGRM. We show that the eGRM better captures the structure of a population than the canonical Genetic Relationship Matrix (GRM), even when using limited genetic information found on a genotyping array. Moreover, the eGRM can reveal the time-varying nature of population structure in a sample. Second, we devised a method called gLike that derives the full likelihood of a genealogical tree under any hypothesized demographic history. Employing a graph-based structure, gLike summarizes the relationships among all lineages in a tree with all possible trajectories of population memberships through time and efficiently computes the exact marginal probability under a parameterized demographic model. Through extensive simulations of multiple admixtures, we showed that gLike accurately estimates dozens of demographic parameters, including ancestral population sizes, admixture timing, and admixture proportions, and outperforms conventional demographic inference methods that leverage only the allele frequency spectrum. We applied both methods to real-world human genomic data from Finnish, Latino American, and Native Hawaiian cohorts to gain further insights into the patterns of population structure and to estimate parameters of the admixture histories. Taken together, our studies demonstrate the power of leveraging the genealogical trees for downstream population genetic inferences.

232 **Creating a fully-differentiable coalescent simulator using StyleGAN** Dylan Ray, Will Booker Genetics, UNC Chapel Hill

Simulation has long been one of the most important tools in population genetics research, enabling us to explore highly entropic distributions of evolutionary histories, involving recombination, mutation, selection, and demographic changes. Simulations aid in the development, testing, and training of tools used to make evolutionary inferences from real data collected from natural populations. While highly versatile and increasingly computationally efficient, population genetic simulations construct a series of inherently discrete events drawn from probability distributions which are in turn conditional upon past events, and thus writing the full likelihood or its derivative with respect to some parameter such as recombination rate or effective population size given a set of inferred genealogies is often cumbersome, analytically intractable, and/or difficult to compute. Given this fact and the high-dimensional nature of genealogies, Approximate Bayesian Computation

with summary statistics is often used in place of the full likelihood function when inferring demographic parameters from real data sampled from natural populations. Here we propose a novel method for a fully differentiable genetic simulator using a Generative Adversarial Network (GAN) that learns a continuous latent space meant to represent a uniformly random mixture of many closely related demographic models, giving an easily sampled distribution with a smooth, fully specified likelihood. We trained a GAN to capture the distribution of single genealogies, show that the resulting GAN is able to compress genealogies efficiently via an easily learnable back-projection, and that the learned latent space and the distribution of replicates it encodes reproduce many of the properties of the original statistical manifold. By mapping regions of latent space to the demographic parameters they were simulated with we define a twice-differentiable likelihood function w.r. to the replicate and any given parameter set within the boundaries, thereby paving the way for the direct estimation of population genetic parameters via gradient descent and broad studies of metrics such as Fisher information which gives the distribution of error for unbiased MLEs. We show that the latent space produced by our GAN can be used to infer population genetic parameters with accuracy comparable to a a recent approach using deep neural networks trained to infer parameter values from population genetic alignments.

233 **Bayesian phylodynamic inference of population dynamics with dormancy** Suvadip Sana[1], Lorenzo Capello[2], Ishani Chopra[3], Wai Tung `Jack' Lo[4], Perry Xu[5], Andrew G. Clark[5], Martin T. Wells[1], Jaehee Kim[3] [1]Department of Statistics and Data Science, Cornell University, [2]Departments of Economics and Business, Universitat Pompeu Fabra, [3]Department of Computational Biology, Cornell University, [4]Department of Computer Science, Cornell University, [5]Department of Molecular Biology and Genetics, Cornell University

Many organisms respond to environmental fluctuations by utilizing reversible dormant states or "seedbanks". This life-history strategy significantly alters the fundamental evolutionary and ecological forces acting on the population, leading to changes in patterns of genetic diversity and population demography. Two fundamentally different models of dormancy have been proposed based on the average time individuals spend in the dormant state in comparison to the evolutionary timescale measured by the coalescent: "weak" seedbank that models dormancy induced by scheduled seasonality (e.g., plants or invertebrate species) and "strong" seedbank where individuals stochastically switch between active and dormant states (e.g., bacteria).

Because the weak seedbank has been shown to be statistically equivalent to the Kingman coalescent with a population-rescaled mutation rate, inference under the weak seedbank coalescent directly extends the existing methods. The strong seedbank coalescent, however, fundamentally differs from existing coalescent models. While it shares similarities with the two-population structured coalescent in that individuals can stochastically transition between states, its key distinction lies in allowing only active state lineages to coalesce, whereas those in the dormant state cannot. This asymmetry in coalescence events presents challenges in applying existing inference methods developed for the structured coalescent. Consequently, despite the significant role of dormancy in the eco-evolutionary dynamics of many organisms, no methods currently exist for inferring population dynamics involving dormancy and associated parameters.

Here, we developed a Bayesian phylodynamic method under the strong seedbank coalescent that enables the joint inference of genealogy, dormancy model parameters, and evolutionary model parameters from genetic data. We 1) provide a genealogical density under the strong seedbank coalescent; 2) prove a new phylogenetic likelihood formula for the strong seedbank tree; 3) propose new tree operators for efficiently exploring the tree space for the strong seedbank tree; 4) validate our method both with synthetic and real data; and 5) implement our inference framework as a package in BEAST2. Our work serves as the groundwork for developing theoretical foundations and more refined inference methods for populations undergoing dormancy.

234 **Tests of Ghost Introgression into Extant Lineages** Margaret Wanjiku, Arun Sethuraman Biology, San Diego State University

Gene flow from unsampled or extinct ghost populations leaves signatures on the genomes of individuals from extant, sampled populations, often introducing biases, data misinterpretation, and ambiguous results when estimating evolutionary history from population genomic data. Here, we utilize extensive simulations under various ghost topologies, with no gene flow to extensive gene flow to and from an unsampled ghost population. These models help us systematically assess biases while accounting or not accounting for gene flow from ghost populations in demographic history (mutation-scaled effective population sizes, divergence times, and migration rates) under the Isolation with Migration (IM) model. Estimates of evolutionary history across all scenarios of deep divergence of an outgroup ghost indicate consistent a) under-estimation of divergence times between sampled populations, (b) over-estimation of effective population sizes of sampled populations, and (c) under-estimation of migration rates between sampled populations, with increased gene flow from the unsampled ghost population.

Additionally, considering the large effects of gene flow from ghost populations, we propose a multi-pronged approach to account for the presence of unsampled ghost populations in population genomics studies to reduce erroneous inferences. We present three statistical tests to test for the presence of an unsampled ghost in population genomic data. They utilize (1) inference of population structure under the admixture model, (2) inference of the distribution of coalescent times across genomic loci, and (3) inference of the goodness of fit under the IM model to test for the presence of an unsampled ghost population among sampled population genomic data. We recommend these tests as a necessary first step before estimating evolutionary history under restrictive (e.g., two population) demographic models.

235 **Stabilizing selection shapes the distribution of shared variation after archaic introgression** Aaron P Ragsdale Integrative Biology, University of Wisconsin-Madison

Genomic studies show that admixture commonly occurs between diverged populations and sometimes between closely related taxa. Human history in particular involves repeated migration, admixture and introgression events. Some genetic segments of present-day populations trace to Neanderthals, Denisovans and potentially other hominin lineages, and there is growing evidence that early *H. sapiens* reciprocally contributed genetic material to Neanderthals. Bidirectional introgression introduced genetic variation that contributed to phenotypic traits in both humans and Neanderthals. Past episodes of selection on trait-affecting alleles have shaped genetic variation in functional genomic regions, leaving footprints that can be observed in the genomes of Neanderthals and present-day populations.

Many phenotypic traits are subject to stabilizing selection, which acts to maintain population-average phenotypic values near some optimum. Drawing on classical theory and recent computational developments for the site-frequency spectrum, we develop a population genetic approach to model genetic and phenotypic variation for traits under stabilizing selection, allowing for complex demographic history involving migration and admixture. We use this to predict the genetic architecture of traits under stabilizing selection after introgression, and we show that stabilizing selection results in selection against introgressed ancestry surrounding functional regions. By contrasting to predictions based on models of deleterious load and genomic incompatibilities, we show that stabilizing selection can instead explain observed co-occurrences of introgressed ancestry deserts in both humans and Neanderthals. Because many traits are under stabilizing selection, we argue that it is a useful null model for studying natural selection and the architecture of complex traits after introgression between diverged lineages.

236 **A machine vision guided robot for fully automated embryonic microinjection** Andrew D Alegria[1], Amey S Joshi[1], Jorge B Mendana[2], Kanav Khosla[1], Kieran T Smith[3], Benjamin Auch[2], Margaret Donovan[2], John Bischof[1], Daryl M Gohl[4], Suhasa B Kodandaramaiah[1] [1]Mechanical Engineering, University of Minnesota, [2]University of Minnesota, [3]Fisheries, Wildlife and Conservation Biology, University of Minnesota, [4]Genetics, Cell Biology, and Development, University of Minnesota

Microinjection is a widely used technique for transgenesis, mutagenesis, cell labeling, cryopreservation, and *in vitro* fertilization in many organisms. Microinjection requires specialized skills for each target organism and involves rate limiting and labor-intensive preparatory steps. Here we constructed a machine vision guided generalized robot that fully automates the process of microinjection in fruit fly *(Drosophila melanogaster)* and zebrafish (*Danio rerio*) embryos. The robot uses machine learning models trained to detect individual embryos in images of agar plates, and models trained to identify specific anatomical locations within each embryo in 3D space using dual view microscopes. The robot uses this information to serially perform microinjection in each detected embryo without any human intervention. We constructed and used three such robots to automatically microinject tens of thousands of Drosophila and zebrafish embryos. We systematically optimized robotic microinjection for each species and validated the use of the robot by performing routine transgenesis with proficiency comparable to highly skilled human practitioners while achieving up to 4x increases in microinjection throughput in Drosophila. The automated microinjection robot was utilized to microinject a pool containing tens of thousands of uniquely barcoded plasmids to rapidly generate more hundreds of unique transgenic Drosophila lines and to carry out a novel measurement of the number of independent germline integration events per successfully injected embryo. Next, we showed that robotic microinjection of cryoprotective agents in zebrafish embryos significantly improves vitrification rates and survival of cryopreserved embryos post-thaw as compared to manual microinjection. We are currently using the robotic microinjection system to perform a large-scale mutational scanning experiment to dissect a large development enhancer region. In the future, we anticipate that this versatile automated microinjection system can be adapted to carry out microinjection in a wide range of other organisms.

237 **The Janelia Atalanta plasmids provide a simple and efficient CRISPR/Cas9-mediated homology directed repair platform for Drosophila** David Stern[1], Elizabeth Kim[2], Emily L Behrman[3] [1]Janelia Research Campus, Howard Hughes Medical Institute, [2]Janelia Research Campus, HHMI, [3]HHMI

Homology-directed repair (HDR) is a powerful tool for modifying genomes in precise ways to address many biological

questions. Use of Clustered Regularly Interspersed Short Palindromic Repeats (CRISPR)-Cas9 induced targeted DNA double-strand breakage has substantially simplified use of homology-directed repair to introduce specific perturbations in *Drosophila*, but existing platforms for CRISPR-Cas9-mediated HDR in *Drosophila* involve multiple cloning steps and have low efficiency. To simplify cloning of HDR plasmids, we designed a new plasmid platform, the Janelia Atalanta (pJAT) series, that exploits recent advances in dsDNA synthesis to facilitate Gateway cloning of gRNA sequences and homology arms in one step. Surprisingly, the pJAT plasmids yielded considerably higher HDR efficiency (approximately 25%) than we have observed with other approaches. pJAT plasmids work in multiple *Drosophila* species and exhibited such high efficiency that previously impossible experiments in *Drosophila*, such as driving targeted chromosomal inversions, were made possible. We provide pJAT plasmids for a range of commonly performed experiments including targeted insertional mutagenesis, insertion of phiC31-mediated attP landing sites, generation of strains carrying a germ-line source of Cas9, and induction of chromosomal rearrangements. We also provide "empty" pJAT plasmids with multiple cloning sites to simplify construction of plasmids with new functionality. The pJAT platform is generic and may facilitate improved efficiency CRISPR-Cas9 HDR in a wide range of model and non-model organisms.

238 **Building metabolic pathway resources for *Drosophila melanogaster*** Steven J Marygold[1], Peter D Karp[2], David P Hill[3], Peter D'Eustachio[4], Jason M Tennessen[5] [1]FlyBase, University of Cambridge, [2]Bioinformatics Research Group, SRI International, [3]The Jackson Laboratory, [4]Department of Biochemistry and Molecular Pharmacology, New York University Grossman School of Medicine, [5]Department of Biology, Indiana University

The ability to study metabolism in *Drosophila melanogaster* relies on having an accurate, comprehensive and up-to-date representation of its metabolic pathways and networks. However, currently available resources (e.g. KEGG, Reactome, FlyCyc) are incomplete/inaccurate as they are based on outdated functional annotations or are generated computationally via orthology. Furthermore, many discrepancies exist between these databases and none of them are integrated with FlyBase, which is the primary source for *Drosophila* functional genomic information and reagents. Therefore, there is an urgent need to establish a set of accurate, canonical metabolic pathways for *Drosophila*, enabled through manual assessment and annotation by expert biocurators.

We are addressing this need using the following approaches. We started by systematically reviewing all known/predicted *Drosophila* enzymes, verifying Gene Ontology (GO) and Enzyme Commission (EC) annotations to >3,500 enzyme-encoding genes within FlyBase. These enhancements then allowed us to compute an updated *Drosophila* metabolic network within the BioCyc database. Compared to the previous version, this revised 'FlyCyc' includes >50 additional metabolic pathways and identifies >600 additional enzyme-encoding genes, thereby providing researchers with much-improved *Drosophila* metabolic pathway diagrams and enhanced capabilities to analyze 'omics data within the BioCyc platform.

We will use the updated FlyCyc, together with orthology-based pathway predictions from Reactome, as a basis to create GO-Causal Activity Model (GO-CAM) representations of *Drosophila* metabolic pathways. GO-CAMs are created manually by expert curators and are directly linked to the primary GO annotations. This means that each pathway is manually verified with transparent evidence for each reaction, and allows for the creation of tissue-, context- and *Drosophila*-specific versions of metabolic pathways. Moreover, the GO-CAM framework is being employed across Model Organism Databases, making them easily comparable and inter-operable.

The new *Drosophila* metabolic pathway resources will be invaluable for the research community and will be made available through the FlyBase, BioCyc and Alliance of Genomic Resources websites.

239 **The Alzheimer's Disease Fly Cell Atlas (AD-FCA): A Whole-Organism, Single-Cell sequencing resource on Brain-Body Interactions** Ye-Jin Park[1,2,3,4], Tzu-Chiao Lu[1,2], Tyler Jackson[1,2,5], Jiaye Chen[6], Yanyan Qi[1,2], Chung-Yi Liang[1,2,7], Lindsey Ran[8], Erin Harrison[1,2], Christina Ko[8], Madeline Burns[1,2], Hugo J Bellen[1,4], Hongjie Li[1,2] [1]Department of Molecular and Human Genetics, Baylor College of Medicine, [2]Huffington Center on Aging, Baylor College of Medicine, [3]Program in Development, Disease Models & Therapeutics, Baylor College of Medicine, [4]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [5]Program in Cancer Cell Biology, Baylor College of Medicine, [6]Program in Quantitative & Computational Biosciences, Baylor College of Medicine, [7]Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, [8]Neuroscience Program, BioSciences Department, Rice University

Brain aging and neurodegeneration have been extensively investigated, with a predominant focus on the nervous system, but emerging evidence indicates that the effects of brain aging and diseases extend beyond the central nervous system to peripheral organs. Alzheimer's Disease (AD), for instance, has been associated with disruptions in the gut microbiome, respiratory system, cardiovascular function, and hormone regulation, underscoring the involvement of peripheral tissues in

the disease process. However, a comprehensive understanding of how impaired neurons impact peripheral tissues within the broader context of the whole organism has been lacking. To address this gap, we generated the Alzheimer's Disease Fly Cell Atlas (AD-FCA). This atlas profiles single-nucleus transcriptomes of the entire Drosophila organism, comparing normal aging flies with experimental flies where we induce neuronal insults through Abeta42 or Tau transgenes. The AD-FCA characterizes 219 distinct cell types from over 624,000 nuclei and analyzes transcriptomic changes in both the nervous system and peripheral tissues at the cellular level, allowing comparisons between genotypes, ages, and sexes. This pioneering whole-organism AD atlas serves as a valuable resource for the AD research community, facilitating the discovery of progressive changes that likely occur in non-neurological tissues in response to AD, thus offering fresh insights into the realm of brain-body communication.

240 **OligoY pipeline allows full Y chromosome painting assays** Isabela Almeida[1], Henry AB Bruno[1], Mara MLS Pinheiro[1], Antonio B de Carvalho[2], Maria Vibranovski[3,4] [1]Genetics and Evolutionary Biology, University of São Paulo, [2]Genetics, Federal University of Rio de Janeiro, [3]Genetics and Evolutionary Biology, University of Sao Paulo, [4]School of Mathematical and Natural Sciences, Arizona State University

Y chromosomes remain understudied for most species due to their haploid and highly repetitive nature, posing a challenge for assembly algorithms and leading to contig-fragmentation and sequence loss. As a result, the Y chromosome has received little attention from cytogeneticists, even though doing so would allow them to better understand its function throughout the cell cycle and provide new insights into its evolutionary history and relationships with other regions of the genome. Importantly, the conventional protocol for designing probes used in full chromosome fluorescent labeling experiments (FISH Oligopaint) does not include repetitive sequences to avoid off-target hybridization. For the Y chromosome, the remaining non-repetitive sequences are numerically insufficient to design probes and efficiently perform FISH Oligopaint assays.

OligoY pipeline generates FISH Oligopaint probes for the Y chromosome of any species by using a genome assembly and female and male reads to enhance the number of known sequences. The pipeline effectively uses repetitive sequences unique to the target chromosome to design probes, all while guaranteeing the user the autonomy to choose parameters, thus maximizing overall efficiency of cytogenetic experiments. After extensive in silico and in situ tests and validations with *Drosophila melanogaster,* we showed for the first time a pipeline for probe design that significantly increases previous Y chromosome staining with no off-target signal.

241 **PANGEA: A New Gene Set Enrichment Tool for Common Research Organisms** Claire Hu[1], Aram Comjean[1], Helen Attrill[2], Giulia Antonazzo[3], Jim Thurmond[4], Weihang Chen[5], Stephanie Mohr[5], Nicholas Brown[3], Norbert Perrimon[5] [1]Genetics, Harvard Medical School, [2]Physiology, University of Cambridge, [3]University of Cambridge, [4]Indiana University, [5]Harvard Medical School

Over the years, the Drosophila RNAi Screening Center (DRSC) bioinformatics group has implemented a suite of informatics tools (https://fgr.hms.harvard.edu/tools), including gene-centric resources that facilitate ortholog and paralog mapping, and mining of information about orthologs and paralogs in common genetic model species; reagent-centric resources that help researchers identify RNAi and CRISPR sgRNA reagents or designs; and data-centric resources for visualization and mining of transcriptomics data, protein modification data, protein interactions, and more. Our resources are actively updated and expanded based on community input. For example, GuideXpress was recently expanded to support sgRNA design and ortholog search for the blacklegged tick (*Ixodes scapularis*). In addition, we recently launched PANGEA, a new tool for gene set enrichment (GSEA). GSEA plays an important role in large-scale data analysis, helping scientists discover underlying biological patterns over-represented in gene lists resulting from 'omics' or other large-scale studies. Gene Ontology (GO) annotation is the most frequently used classification mechanism for gene set definition. PANGEA (PAthway, Network and Gene-set Enrichment Analysis; https://www.flyrnai.org/tools/pangea/), allows a more flexible and configurable approach to GSEA using a variety of classification sets. PANGEA supports GO analysis using different sets of GO annotations, such as the set of GO annotations that exclude results from high-throughput studies. PANGEA also supports GSEA with pathway annotations and protein complex datasets from various resources, as well as gene expression and disease annotations from the Alliance of Genome Resources. Visualizations include an option to view a network of gene set-to-gene relationships, and comparisons of multiple input gene lists. PANGEA will facilitate GSEA for *Drosophila, C. elegans,* zebrafish, and mammalian genes (human, mouse, and rat) based on high-quality annotated information available for these species. Altogether, our established and new resources support research for major model organisms at all stages of the research pipeline, from development of candidate gene lists and identification of reagents to data analysis, visualization, and integration.

242 **The genetic basis of novel cell type evolution in the *Drosophila* sex comb** Ben Hopkins[1], Olga Barmina[2], Artyom Kopp[3] [1]Evolution & Ecology, University of California – Davis, [2]Evolution & Ecology, University of California, Davis, [3]University of California, Davis

The evolution of new cell types has been a major contributor to the diversification of animal life and underlies many of its key innovations, from the immune system to the brain. However, our understanding of the genetic mechanisms through which new cell types originate remains limited. Arguably, the most conspicuous novel cell types in *Drosophila melanogaster* are those of the sex comb, a recently evolved, male-specific structure found on the foreleg tarsus of species in the *Sophophora-Lordiphosa* radiation. We know that the sex comb evolved through rewiring the differentiation program that builds mechanosensory bristles by linking it to the expression of *doublesex*. However, the identities of the downstream genes that drive this transformation and the mode of gene expression evolution responsible remains unknown. Is the transformation driven by quantitative or heterochronic changes in the expression of genes that are shared with mechanosensory bristles? Or by the novel gain of expression of genes that aren't active in the mechanosensory bristles? Using a time-series single-cell RNA-seq dataset of the developing tarsus, coupled with hybridisation chain reaction (HCR) in situs, we show that the cells of the sex comb exhibit elements of all three modes of gene expression evolution. The bristle-building genetic program is initiated earlier in sex comb cells than in the surrounding mechanosensory bristles, conserved elements of this program are upregulated relative to their expression in the surrounding mechanosensory bristles, and the expression of several genes that aren't expressed in tarsal mechanosensory bristles, including *b* and *Pxd*, appears to have been gained in sex comb cells. We further show that the deployment of *b* and *Pxd* expression in bristle cells isn't unique to the sex comb, but rather a feature of several large bristle and bristle-like structures elsewhere in the fly, such as the preapical bristles, wing pegs, and genital claspers. It therefore appears that the sex comb evolved, at least in part, through the redeployment of an existing gene module in a new cellular context. We also show that this gene module appears to be active in the sex comb cells of other *Drosophila* species from across the *Sophophora* subgenus, suggesting that the genetic basis of sex comb tooth development is largely conserved.

243 **Comparative developmental genetics of female-limited mimicry polymorphisms** Sofia I. Sheikh[1], Nicholas W VanKuren[2], Meredith M Doellman[2], Phoebe Hall[2], Marcus Kronforst[2] [1]Ecology and Evolution, University of Chicago, [2]University of Chicago

Sex-limited polymorphisms, such as damselfly female color morphs and butterfly mimicry polymorphisms, are frequently both adaptive and drive phenotypic diversification, yet the molecular mechanisms by which the development of multiple complex phenotypes can be limited to one sex remain unclear. Considerable work has been done exploring the ecological pressures and evolutionary processes that necessitate and maintain genetic variation resulting in multiple adaptive morphs. However, very little is known about the developmental genetics of discrete phenotypic morphs. One powerful system to investigate this is the *Papilio* swallowtail butterflies, in which multiple closely related species have evolved a female-limited mimicry polymorphism (FLMP) wherein females develop either mimetic or non-mimetic wing color patterns. While the color patterns of the morphs are strikingly different across species, each polymorphism is regulated by allelic variation at *doublesex* (*dsx*). How and why this gene has been repeatedly involved in the evolution of sex-limited polymorphisms remains unclear. Across three species with FLMP, we found that in each case both the mimetic and non-mimetic females develop male-like color patterns when we knockdown Dsx expression with RNAi, suggesting that this gene controls both sexual dimorphism and female-limited polymorphism. We also found that the derived *dsx* allele in the species *P. lowii* has acquired unique spatiotemporal expression patterns as shown by antibody staining. Moreover, these patterns differ from how *dsx* is expressed in the mimetic females of the closely related species *P. alphenor.* To uncover the downstream genes involved in the color pattern switch, we used RNA-seq and compared the results to previous work in *P. alphenor*. Although some genes are DE in both species, the temporal pattern of differential expression is notably different, suggesting distinct mechanisms of the *dsx* switch function. Lastly, we paired Dsx antibody staining with other candidate gene expression using HCR to determine how *dsx* modifies existing gene regulatory networks to drive the production of distinct color patterns. Our results indicate that, despite the shared use of *dsx*, the functional basis of female-limited mimicry polymorphism appears to be different between *P. lowii and P. alphenor*. These results indicate that deeply conserved developmental pathways are highy labile, permitting multiple underlying functions to arrive at the same state of sex-limited polymorphism in closely related species.

244 **Chromatin Dynamics and Aging in *C. elegans*** Siu Sylvia Lee Molecular Biology and Genetics, Cornell University

We are interested in understanding the interplay between chromatin dynamics, gene expression, and aging. We use *C. elegans* as a model to dissect how specific histone-modifying factors modulate longevity and explore how the global chromatin dynamics change with aging. We demonstrated that the chromatin factors SET-26 and HCF-1 act in the soma to modulate stress responses and longevity. We revealed that SET-26 localizes to chromatin by binding to the histone mark H3K4me3, subsequently recruiting HCF-1. Additionally, we identified the histone deacetylase HDA-1 playing an antagonizing role to SET-26/HCF-1. Interestingly, HDA-1 localizes to genomic regions closely aligned with the SET-26 and HCF-1 binding sites but is recruited to the chromatin independently of SET-26 or HCF-1. Gene expression profiling unveiled that SET-26/HCF-1 and HDA-1 exert opposing effects on the expression of specific target genes, which likely contribute to their antagonistic roles in longevity. To further comprehend how specific chromatin perturbations impact aging, we have probed the age-dependent dynamics of several key histone modifications. We found that while the repressive mark H3K27me3 remains stable with age,

the heterochromatin mark H3K9me3 exhibits significant redistribution, displaying both global loss and local gain. Additionally, although the active histone modification H3K36me3 remains stable with aging, the levels of H3K36me3 have a causal relationship with the age dynamics of gene expression. Future studies will delve into the functional implications of these changes in determining longevity.

245 **Vitamin B12 Protects Against DGLA-Induced Ferroptosis in *C. elegans*** Michael S Mortensen, Jennifer L Watts School of Molecular Biosciences, Washington State University

Ferroptosis is a novel form of regulated cell death characterized by membrane destruction brought about by iron-dependent lipid peroxidation of polyunsaturated fatty acids (PUFAs). Since its discovery in 2012, ferroptosis has been implicated in various biological processes and conditions, including tumor suppression, aging, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Our lab discovered that the omega-6 PUFA, dihomo-gamma-linolenic acid (DGLA), is capable of inducing sterility in the nematode, *Caenorhabditis elegans* (*C. elegans*), through triggering ferroptosis specifically in the germ cells of young animals. Furthermore, DGLA is also sufficient to induce ferroptosis in human cancer cells, marking this PUFA as a ferroptotic trigger across models. Our research group recently identified that DGLA has a greatly diminished effect in *C. elegans* fed on bacteria containing a higher level of vitamin B12 compared to their standard bacterial food source. Furthermore, we uncovered that dietary vitamin B12 supplementation suppresses DGLA-induced ferroptosis in *C. elegans* in a dose-dependent manner. Vitamin B12 works as a cofactor to the methionine synthase enzyme that functions within the methionine cycle, which is a process revolving around methyltransferase production. We found the loss-of-function mutant for the methionine synthase ortholog in *C. elegans*, *metr-1*, elicits a severe sterility response to DGLA, even on bacterial strains with adequate levels of vitamin B12. Furthermore, *metr-1* worms are not rescued by dietary vitamin B12 supplementation when cotreated with DGLA, indicating that vitamin B12 protects from DGLA-induced ferroptosis directly through its role as a cofactor in the methionine cycle. These findings highlight a role for protection against ferroptosis dependent on vitamin B12 and its function in the methionine cycle.

246 **Neurobehavioral and mobility phenotypes of adenylosuccinate lyase deficiency are due to distinct mechanisms of SAICAR toxicity** Sabrina Amita Sony, Corinna A Moro, Latisha P Franklin, Mia M Peifer, Wendy Hanna-Rose Biochemistry and Molecular Biology, The Pennsylvania State University

Adenylosuccinate lyase (ADSL) deficiency is a rare disorder that causes muscle dysfunction, behavioral abnormalities, and autistic-like symptoms. Deficiency of this purine biosynthetic enzyme leads to the buildup of substrate SAICAR and a disruption in the homeostasis of purines. Each effect is thought to contribute to generating the phenotypes, but the mechanisms linking metabolic effects to behavioral symptoms are unknown. We use *C. elegans* to investigate the neurobehavioral functions of *adsl-1.*

adsl-1(RNAi) animals exhibit slow and uncoordinated movement, an increased tendency to reverse and a novel learning phenotype. Reduction of *adsl-1* function does not prevent learning but causes a distinct behavioral output after cue-pairing in a gustatory plasticity paradigm. To determine the effect of distinct metabolic changes on phenotypes, we assessed phenotypes after supplementing cultures with excess purines and after manipulating SAICAR levels via pharmacological approaches. Our results indicate that increased SAICAR levels contribute to both learning and mobility phenotypes. In contrast, purine homeostasis does not appear to underlie the mobility phenotypes. However, supplementation with adenosine, but paradoxically not guanosine, can partially restore learning.

Metabolomics analysis revealed that tyrosine metabolism is perturbed when *adsl-1* function is reduced, which led us to discover that the learning difference is a result of a deficiency of the tyrosine-derived biogenic amine tyramine. Loss of TDC-1, which synthesizes tyramine, or the TYRA-2 tyramine receptor, causes an *adsl-1*-like learning difference, and the learning phenotype of both *adsl-1* and *tdc-1* is ameliorated with tyramine supplementation. The working model that reduced *adsl-1* function inhibits tyramine production is also supported by the observation that *adsl-1* mutants manifest the distinct head oscillation phenotype seen in *tdc-1* mutants. While tyramine supplementation restores learning, it has no effect on mobility of *adsl-1* animals, suggesting that the mechanisms by which SAICAR influences mobility and learning are distinct.

Finally, we have shown that the restorative effect of adenosine on learning does not extend to the head oscillation phenotype, suggesting that we have revealed an unexpected role for adenosine directly in gustatory plasticity as opposed to in ameliorating the metabolic effects of reduced *adsl-1* function on tyramine availability.

Keywords: Purine Metabolism, ADSL Deficiency, *C. elegans*, Behavior, Tyramine

247 **Nutrient-Induced Overcoming of Dual Inhibition on MYRF Cleavage on the Cell Membrane is essential for Temporal Developmental Control in *C. elegans*** Zhimin Xu, Zhao Wang, Shiqian Shen, Lifang Wang, Yingchuan B Qi ShanghaiTech

University

The regulatory principles governing post-embryonic development, especially those related to timing control, remain largely elusive. MYRF, a transmembrane transcription factor, plays a pivotal role in *C. elegans* larval development, and its absence results in early larval arrest. MYRF is well-known as a master regulator of myelination. However, its presence across metazoans and widespread expression hints at functions extending beyond myelination. Loss of MYRF results in developmental lethality in both invertebrates and vertebrates, and in humans, MYRF haploinsufficiency is linked to *MYRF*-related Cardiac Urogenital Syndrome. Despite its importance, the mechanisms underlying MYRF's involvement in animal development remain largely uncharted.

MYRF stands out as an unconventional transcription factor, which is initially inserted in the membrane, and then undergoes intramolecular chaperone-mediated trimerization. This trimerization triggers self-cleavage, allowing its N-terminal segment to enter the nucleus for transcriptional regulation. We previously observed that full-length MYRF traffics to the cell membrane in *C. elegans*, and the self-cleavage is temporally controlled by yet-to-be-identified mechanisms.

In this study, we unveil that the proteolytic processing of MYRF is negatively regulated by dual inhibitory interactions, involving both MYRF itself and a second transmembrane factor, PAN-1. Releasing these inhibitions is crucial for self-cleavage. The processing of MYRF oscillates with the four consecutive stages of larval development, likely influenced by the internal nutrient state, as discontinuing feeding effectively blocks MYRF's cleavage.

To overcome the early lethal consequences of MYRF deletion, we established a system for the inducible degradation of MYRF protein. Our findings show that MYRF plays a vital role at each larval stage, operating within specific, short time frames. This reveals its broad yet temporally specific involvement in developmental progression. Notably, MYRF is responsible for promoting the transcriptional activation of the key developmental timing gene miRNA *lin-4*.

These findings unveil a previously uncharted pathway that connects the overall nutrient status to transcriptional developmental programs.

248 **Loss of the H3K9me2 demethylase *jhdm-1* in *C. elegans* results in a transgenerational decrease in chemotaxis** Jaely Z Chavez[1], Mackenzie S Roberson[1], Monica Reeves[2], Karen L Schmiechel[1], David J Katz[2] [1]Biology, Oglethorpe University, [2]Cell Biology, Emory University

Recently in *Caenorhabditis elegans*, we showed that animals mutant for the COMPASS H3K4 methyltransferase complex member *wdr-5,* experience a global transgenerational enrichment of repressive H3K9me2 marks. The accumulation of H3K9me2 in these animals is associated with a progressive longevity phenotype where animals achieve significantly longer lifespans with increasing generations. The importance of the management of H3K9-dependent heterochromatin is further underscored by the fact that this expanded longevity can also be recapitulated by inhibition of the putative H3K9me2 demethylase JHDM-1. To determine whether the accumulation of H3K9me2 affects behavior, we performed chemotaxis assays on *jhdm-1* mutants. We find that *jhdm-1* mutants exhibit significantly reduced chemotactic behavior towards food in comparison to wild-type N2 worms. Consistent with this being an epigenetic effect, the chemotaxis index (C.I.) in *jhdm-1* mutant worms declines transgenerationally by 52.5% between generations F3 and F7 ($C.I._{F3}$=0.80 and $C.I._{F7}$= 0.43). To our knowledge, this is the first example of a transgenerational behavior phenotype potentially caused by the accumulation of histone methylation across generations. Interestingly, after generation F7, *jhdm-1* mutants gradually restore their chemotactic behaviors to levels comparable to those seen in early generations ($C.I._{F13}$= 0.86). The transgenerational decline in chemotactic behavior and the subsequent reversion of the phenotype in *jhdm-1* mutants is phenocopied in *wdr-5* mutants, which also aberrantly accumulate H3K9me2. Taken together, these data are consistent with the interpretation that transgenerational establishment of repressed chromatin through the accumulation of H3K9me2 can result in abnormal behavior in *C. elegans*. We are currently performing RNA-Seq and analyzing H3K9me2 ChIP data to test this hypothesis.

249 ***lon-1* as a nexus of both positive and negative transcriptional outputs of BMP signaling in *C. elegans*** Maria V Serrano, Sergio Moreira-Antepara, Jun Liu Cornell University

Bone Morphogenetic Protein (BMP) signaling regulates a wide variety of developmental and homeostatic processes. However, very few direct target genes have been identified and characterized. The BMP pathway is conserved in *C. elegans* and is known to regulate both body size and postembryonic mesoderm development. Previous work has suggested that *lon-1* transcription is negatively regulated by BMP signaling (Morita et al 2002, Maduzia et al 2002). *lon-1* encodes a protein in the CAP (Cysteine-Rich Secretory Protein, Antigen 5, and Pathogenesis-Related 1) superfamily, and *lon-1(0)* mutants are long.

Using two complementary approaches, we have found that *lon-1* expression is likely directly regulated by BMP signaling, both

positively and negatively. Using a single copy *lon-1* transcriptional reporter system, we identified a region in the *lon-1* promoter that mediates its response to BMP signaling. CHIP-seq data generated by the modENCODE consortium shows that this region is bound by both SMA-3, a Smad protein, and SMA-9, a zinc finger transcription factor and homolog of Drosophila Schnurri. When we deleted this region in the endogenous *lon-1* promoter using CRISPR, we found that *lon-1* function is compromised, exhibiting phenotypes in both body size and mesoderm development in sensitized genetic backgrounds. We have also used an endogenously tagged and fully functional V5::LON-1 to monitor LON-1 protein expression in various BMP pathway mutants. Results from these analyses indicated that *lon-1* expression is regulated both positively and negatively by BMP signaling, and that the regulation exhibits developmental stage specificity. These findings highlight the complex and redundant regulatory mechanisms underlying BMP signal transduction that ensures the robustness of development and homeostasis.

250 **The origin and evolution of de novo genes and de novo proteins** Li Zhao The Rockefeller University

Understanding the origin and evolution of genes is crucial for explaining the origin and evolution of novel phenotypes. While most new genes derive from duplication-related processes, recent studies show that de novo genes, originating from ancestrally non-genic sequences, also contribute to gene and functional innovation. However, the initial steps in the birth process of de novo genes and their maintenance in populations and species were largely unknown. We combined population genetics, whole-genome alignments, computational structure modeling, and functional approaches to study the origination, evolution, and protein structures of lineage-specific de novo genes. We observed a gradual shift in some of their functional and structural properties. Intriguingly, minimal protein structural changes occurred in both young and old de novo genes. Ancestral sequence reconstruction showed that well-folded proteins often originate as folded, some enriched with transmembrane and signal proteins. We also used ATAC sequencing, single-cell RNA sequencing, and deep learning to investigate the regulatory dynamics and mechanisms of these novel genes. Our study provides a systematic overview of the origin, evolution, expression regulation, and structural changes of de novo genes.

251 **A vast evolutionarily novel translatome participates in conserved cellular processes** Aaron C Wacholder[1], Saurin Parikh[1], Nelson Coelho[2], Carly Houghton[2], Lin Chou[2], Anne-Ruxandra Carvunis[1] [1]Computational and Systems Biology, University of Pittsburgh, [2]University of Pittsburgh

Ribosome profiling experiments demonstrate pervasive translation of eukaryotic genomes outside of annotated coding sequences. This “noncanonical” translatome encodes thousands of proteins that potentially participate in biological processes but have never been studied in depth. We developed a novel approach to identify the noncanonical translatome at high sensitivity by integrating ribosome profiling reads across hundreds of published experiments. Applying this approach *to S. cerevisiae*, we identified a yeast translatome consisting of over 18,000 noncanonical coding sequences in addition to around 6000 annotated protein-coding genes. To better understand the role of noncanonical translation, we then characterized the yeast translatome by evolutionary properties and phenotype.

Using comparative genomics, we find that the yeast translatome can be divided into two classes that can be defined evolutionarily. The smaller class consists of coding sequences that are conserved over long evolutionary time; these are mostly annotated and tend to be long and highly expressed. The larger class consists of evolutionarily young coding sequences that emerged recently from noncoding sequences; most are short, relatively lowly expressed and unannotated. Employing a genetic screen in which translation is experimentally disabled, we find that a large fraction of both canonical and noncanonical recently-emerged coding sequences show substantial reductions of fitness despite lacking evolutionary conservation. Moreover, we find that the potential roles of a subset of recently-emerged ORFs can be inferred from their interactors in the yeast genetic interaction network. Novel ORFs are rapidly integrated into conserved cellular networks, and appear to be involved in diverse processes including RNA processing, DNA repair and cell growth.

Overall, our results demonstrate the existence of thousands of evolutionarily unconserved coding sequences, most of which are unannotated and have never been studied, that appear to play key roles in major biological processes.

252 **Evolutionary Signatures of Host-Pathogen Interactions through the Lens of Paleogenomics** Flavio De Angelis, C. Eduardo Guerra Amorim California State University Northridge

Exposure to pathogens stands as one of the most powerful selective pressures in human history. Large-scale genome-wide association studies (GWAS) have illustrated that the genetic predisposition to infections, as well as resistance against pathogens, arise from the contribution of thousands of loci across the genome. To model the polygenic inheritance of these traits, one can integrate the effects of multiple variants into polygenic risk scores (PRS), offering an estimate of an individual’s genetic potential for a trait. Based on this, we sought to calculate immune-related PRS for over 3,000 ancient human individuals, in order to characterize the evolutionary signatures of human host-pathogen interactions during past epidemics in Eurasia. We hypothesize that major epidemics imposed selective pressure on genetic variants underlying

resistance and predisposition to infectious diseases, modeled here as polygenic traits, and that this process has likely left a discernible signature in the genome, detectable through the calculation of PRS for ancient individuals across different epochs. By combining GWAS with paleogenomic data, we show that major epidemics in human pre- and historical eras have shaped the genetic variation associated with polygenic traits that underlie infectious disease susceptibility. Notably, five events – the transition to agriculture, Justinian Plague, Antonian Plague, Black Death, and Measles outbreaks – are associated with statistically significant shifts in the distribution of PRS calculated based on these traits. Among the biological processes implicated in the adaptation to disease outbreaks are lipid metabolism and lipoprotein clearance, the differentiation and development of megakaryocytes, and the response to hormonal stimuli, particularly insulin, emphasizing systemic involvement in host defenses.

253 **Horizontal gene transfer as a mechanism for evolution of novel immune defenses in Drosophila** Rebecca Tarnopol[1], Josephine A Tamsil[2], Jaden Ha[2], Gyöngyi Cinege[3], Zoltan Lipinzski[4], Bernard Y Kim[5], Lilla B Magyar[3], Kirsten I Verster[5], Éva Kurucz[3], Edit Ábraham[4], Susan L Bernstein[2], István Andó[3], Noah K Whiteman[2,6] [1]Plant & Microbial Biology, UC Berkeley, [2]Molecular & Cell Biology, UC Berkeley, [3]Institute of Genetics, Biological Research Centre -- Szeged, [4]Institute of Biochemistry, Biological Research Centre -- Szeged, [5]Biology, Stanford University, [6]Integrative Biology, UC Berkeley

Immune systems are among the most dynamically evolving traits across the tree of life. Even with a limited immune repertoire, insects have evolved unique defense strategies to neutralize parasitoid wasps, which impose some of the strongest selection on insect populations in the wild. Studies of *Drosophila* parasitoid defense systems have focused on cell-mediated defense systems, where the parasitoid is encapsulated by host blood cells and neutralized. We previously discovered that *Drosophila ananassae* possesses a non-canonical immune response to parasitoids that involves two toxin genes, cytolethal distending toxin B (*cdtB*) and apoptosis inducing protein of 56kDa (*aip56*), that were horizontally transferred from phages or bacteria to the ancestor of the *ananassae* group ~21 mya. While these genes are necessary for *D. ananassae* to mount a full immune response against parasitoid wasps with diverse infection strategies, a direct role for these toxins in parasitoid defense is unknown. Here, we recapitulated the horizontal transfer of *cdtB* and *aip56* into flies by expressing *cdtB* and two *cdtB::aip56* "fusion" genes from *D. ananassae* in transgenic *D. melanogaster*, which does not natively encode *cdtB* or the *fusion* genes. We found that one widely conserved *cdtB::aip56* fusion gene (*fusionB*) promotes fly survival and suppresses wasp development when expressed in *D. melanogaster* fat bodies. *fusionB* arrests fly development when expressed constitutively, suggesting constraints evolved on its expression in its native hosts. Heterologous expression experiments in yeast indicate that the fusion genes have evolved eukaryotic signal peptides that are essential to the growth inhibition phenotype we observe in eukaryotic hosts. Our findings demonstrate that horizontal gene transfer can promote the rapid evolution of innate immune modules in animal hosts.

254 **Evolutionary diversification and repeated gene capture by telomeric retrotransposons across the *Drosophila* genus** Jae Hak Son[1], Christopher E. Ellison[1], Mia T. Levine[2] [1]Department of Genetics, Human Genetics Institute of New Jersey, Rutgers, The State University of New Jersey, [2]Department of Biology, Epigenetics Institute, Penn Center for Genome Integrity, University of Pennsylvania

Transposable elements (TEs) are mobile genetic elements that can move from one position in the host genome to another and must replicate faster than their host to avoid extinction. TEs often evolve antagonistically with their host in a classic evolutionary arms race. On the other hand, the co-evolution between TEs and the host genome can be mutualistic, where TEs are co-opted to benefit their host. Telomere-specialized non-LTR retrotransposons in *Drosophila* have been known for their mutualistic relationship with their host. These elements have replaced the role of telomerase, which is absent across the *Drosophila* genus, and replicate specifically to chromosome ends to protect them from erosion. However, more recent work has identified rapid evolution in many telomere-binding proteins, which is more consistent with antagonistic evolution rather than mutualism. We recently found that the *D. melanogaster TART-A* telomeric transposon has captured a portion of the piRNA gene, *nxf2*, which allows it to target *nxf2* for suppression, again consistent with antagonistic evolution. To reveal whether the antagonistic evolution between the telomeric transposons and the host occurs across the *Drosophila* genus, we have examined the evolutionary diversification of telomere-specialized retrotransposons across the *Drosophila* genus using the long-read genome assemblies from 109 *Drosophila* species. Based on phylogenetic analysis of the *pol* gene, we identify 6 major telomeric retrotransposon clades. Telomeric TEs exhibit a complex pattern of gain, loss, duplication, and horizontal transfer within and between these clades. The ancestral clade (TR1) has been lost in the *melanogaster* group and the HTT clade, first described in *D. melanogaster*, replaced the ancestral clade in the group most likely via horizontal transfer. We also found that 13 *Drosophila* species independently lost all telomeric retrotransposons. We next find repeated capture of piRNA effector proteins by telomeric TEs, Piwi in the *montium* subgroup and Aub in the *willistoni* and *Zaprionus* groups. The capture of an *aub* fragment appears to have occurred independently at least four times within the *Zaprionus* subgroup. By sequencing small RNAs in a subset of these species, we also find that piRNAs from the gene-captured region of the telomeric TEs are likely targeting Piwi/Aub for suppression. We therefore propose that gene capture by telomeric TEs represents a form of counter-

silencing by TEs against their host.

255 **Duplication, transposition, and divergence of the telomerase RNA underlies the evolution of Mimulus telomeres** Surbhi Kumawat, Jae Young Choi University of Kansas

One of the most fascinating phenomena in evolutionary biology is the rapid evolution of molecular complexes with conserved functions across the tree of life. Studying these complexes have led to novel insights of function and evolution in universally important molecular systems. Here we present results from studying the evolution of the telomere, a nucleoprotein complex that protects chromosome ends in all organisms with a linear chromosome.

Telomere has a crucial role of genomic stability and protection, hence components of the telomere are thought to be under strong evolutionary constraint. In vertebrates, for instance, the DNA sequence of the telomere has not changed for 500 million years, but in that same time plant telomeres have evolved an enormous range of sequence variation. It is fascinating that plant telomeres have deviated from the ultra-constrained evolution, but what mechanism drives the sequence variation is largely unknown. Here, we present results from Monkeyflower (Mimulus) telomeres and propose a novel model to explain telomere sequence variation in plants.

We investigated the evolution of Mimulus telomeres by studying the long noncoding telomerase RNA (TR), which is a core component of the telomere maintenance complex and determines the telomere DNA sequence. We conducted total RNA-based de novo transcriptomics and genome analysis on 22 species, and discovered Mimulus species have evolved at least three different telomere sequence variation. Unexpectedly, we discovered several species with evidence of duplicated TR genes. This was surprising as the telomere maintenance complex uses a single TR gene to extend chromosome ends, hence any extra TR copies might interfere with normal telomere maintenance functions. However, we discovered a Mimulus species (M. lewisii) with two divergent TR duplicates that were functional and resulted in the species with a sequence heterogeneous telomere. Interestingly, evolutionary analysis of the M. lewisii TR paralogs indicated it had arisen from a transposition-mediate duplication process. Further analysis of the TR across multiple Mimulus species we discovered the TR gene had frequently transposed and inserted into new chromosomal positions during the evolution of the Mimulus genus.

We propose the TR transposition, duplication, and divergence model to explain telomere sequence evolution in Mimulus and all plants. This novel model posits complex evolutionary changes in the TR involving transposition-mediated gene duplication results in plant species to evolve telomeres with mixed sequences. These species eventually revert to a single telomere sequence, which facilitates the turnover of the telomere sequence.

256 **Selective dynamics of interruptions at short tandem repeats** Michael E. Goldberg[1], Harriet Dashnow[1], Kelley Harris[2,3], Aaron R. Quinlan[1] [1]Departments of Human Genetics and Biomedical Informatics, University of Utah, [2]Department of Genome Sciences, University of Washington, [3]Computational Biology Division, Fred Hutchinson Cancer Research Center

Short tandem repeats (STRs) are hotspots of genomic instability that mutate at rates orders of magnitude greater than non-repetitive genomic loci. In the germline, STRs typically mutate through replication slippage, which manifests as expansions or contractions of a locus by one or more repeat units. Somatic and germline expansions at some STR loci are linked to Mendelian diseases, while variation at other noncoding loci have recently demonstrated associations with complex traits, possibly by altering transcription factor affinity and occupancy of nearby binding sites. Accordingly, some STRs are inferred to be under purifying selection, regardless of their instability.

A major determinant of an STR's mutation rate is the homology of its repeat units; one or more 'interruptions', or bases that disrupt the locus's canonical repeat, have been shown to significantly decrease mutation rate in both the germline and the soma; the effect size of this decrease scales with the greatest remaining stretch of perfect homology. More broadly, interruptions may act as *cis*-acting anti-mutator alleles, in perfect linkage disequilibrium with the repeat allele whose mutation rate they decrease. However, interruptions may themselves be deleterious at these constrained loci, particularly at noncoding loci in gene regulatory elements, possibly disrupting the formation of secondary structures key to their function. We therefore hypothesized that the frequency of interruptions could depend on a locus's purifying selection, where the fitness effects of both expansions but also interruptions could be more deleterious than at neutral loci.

To test this hypothesis, we examined the distribution of interruptions at nearly 650,000 autosomal 2-6 bp motif STRs. We find that STRs within enhancers and promoters, loci putatively under selection, harbor fewer interruptions than those falling outside. Similarly, STRs are less interrupted when they fall within enhancers linked to highly constrained genes compared to those connected to more neutrally evolving genes. In contrast, purifying selection is positively associated with interruptions in coding STRs. Our findings indicate that the abundance of interruptions may be partially explained at coding STRs by the benefit

of a lower mutational burden at their linked loci. In contrast, maintaining a minimum core stretch of homology may be critical for the function of noncoding STRs that fall within regulatory elements while outweighing the benefits of lowering mutation rate.

257 **Dusky-like shapes the corneal lens by maintaining apical expansion of retinal cells and establishing a scaffold of ZP domain proteins** Neha Ghosh, Jessica E Treisman Cell Biology, NYU School of Medicine

Apical extracellular matrix (aECM) assumes distinct shapes to mediate functions such as morphogenesis, hearing, and fertility. Zona Pellucida (ZP) domain-containing proteins promote aECM attachment to cell membranes, potentially controlling its shape. The *Drosophila* corneal lens is a biconvex structure composed of aECM. We have found that *duskylike* (*dyl*), which encodes a transmembrane ZP domain protein, is essential for normal corneal lens shape; in *dyl* mutant ommatidia, external parts of the corneal lens structure are lost and its internal curvature is increased. During pupal development, loss of *dyl* from the corneal lens-secreting cone and primary pigment cells causes their apical constriction and apicobasal contraction, accompanied by changes in the distribution of actomyosin and β_H-spectrin. We infer that Dyl maintains the apical expansion of these cells by attaching their apical plasma membranes to the aECM under tension. Loss of these attachments in the absence of Dyl may result in ommatidial contraction that pulls the corneal lens basally, altering its curvature. Consistent with this hypothesis, artificial induction of apical constriction by expressing a constitutively active form of Myosin light chain kinase is sufficient to produce the same changes in corneal lens shape seen in *dyl* mutants. Dyl is only transiently expressed in the mid-pupal retina; however, we found that it affects the organization of other ZP domain proteins such as Dumpy (Dpy) and Piopio (Pio) that are maintained until adulthood. These ZP domain proteins have a functional role, as *dpy* mutant ommatidia also show defects in corneal lens shape. Chitin, a major corneal lens component, is deposited above the retinal cells and below a layer of Dpy and Pio, and its accumulation is delayed in both *dyl* and *dpy* mutant ommatidia. These observations suggest that Dyl assembles a scaffold of proteins including Dpy and Pio that retains secreted chitin to establish the normal corneal lens shape. Our work provides mechanistic insights into how the corneal lens develops the curvature necessary to focus light onto the retina.

258 **A novel protein Outspread determines organ dimension during salivary gland tubulogenesis** Ji Hoon Kim, Shravan Balasubramaniam, Parama Paul, Deborah J Andrew Johns Hopkins University

Epithelial tubular organs are essential for viability in all higher multicellular organisms. The optimal functionality of a tubular organ demands its proper architecture, which is achieved during development. Salivary gland (SG) development in the Drosophila embryo provides an excellent model system to study morphogenesis from a 2-dimensional (2D) epithelial sheet into a 3-dimensional (3D) epithelial tube. This process is driven by morphogenetic signals acting on non-muscle myosin II (MyoII) to induce changes in cell shape and arrangement. We have searched for genetic factors that affect SG morphogenesis and found that a novel gene – *outspread* (*osp*) – is important for shaping the 3D structure of the SG. *osp* expression in the SG requires the FoxA transcription factor Fork head (Fkh), which controls SG cell invagination and tube formation. Osp is a large cytosolic protein with two pleckstrin homology (PH) domains that could mediate lipid binding and membrane association. Osp is closely related to a mammalian protein – Myosin Phosphatase Rho Interacting Protein (MPRIP) – suggesting a potential function in the Rho GTPase-MyoII signaling pathway. Indeed, endogenous Osp protein colocalizes with apical MyoII in invaginating SG cells and, when overexpressed, Osp strongly localizes to the supracellular MyoII cable that encircles the SG placode. Loss of *osp* results in shorter SG tubes with wider lumens and Osp overexpression results in longer SG tubes. Similar changes in SG tube dimensions result from perturbing MyoII function: hyperactivation of MyoII decreases SG tube length whereas MyoII depletion specifically in the SG generates elongated SG tubes. Interestingly, *osp* genetically interacts with *arc*, a regulator of Crb, which antagonizes MyoII function in the SG, to aggravate SG morphological defects. Based on these findings, we propose that Osp suppresses MyoII activity during SG invagination thereby providing a novel regulatory mechanism to determine organ geometry during tubular morphogenesis.

259 **The pioneer transcription factor *zelda* is crucial for tissue patterning and morphology in the regenerating *Drosophila* wing imaginal discs** Anish Bose, Keaton Schuster, Chandril Kodali, Rachel Smith-Bolton University of Illinois at Urbana Champaign

The *Drosophila* wing imaginal disc, the larval precursor of the adult wing, is capable of regeneration. During wing disc regeneration, the damaged tissue proliferates and organizes cell fates to re-pattern the tissue to form the adult wing. However, the process behind the re-establishment of cell fate during regeneration as well as the progression from the regenerative state back to normal development is poorly understood. In this study, we show that the pioneer transcription factor Zelda has a novel role in re-patterning the regenerating tissue, allowing it to transition back to normal development and progress through morphogenesis. While Zelda is expressed in the developing wing disc, it is not required for normal wing development. By contrast, Zelda is upregulated after damage, and optogenetic inactivation or RNAi knockdown of Zelda resulted in various cell fate and patterning defects such as 1) missing and/or ectopic veins, 2) thick vein tissue on the distal edge, 3) missing

wing margin, 4) posterior-to-anterior cell fate transformations, and 5) blisters. To identify genes that are regulated by Zelda during regeneration, we performed CUT&RUN to identify Zelda binding sites. Interestingly, Zelda had very few binding sites in undamaged discs but was bound near genes important for wing development during late regeneration. Indeed, we found that inactivation of Zelda resulted in a delayed transition from regeneration to normal developmental patterning, and mis-patterning of genes such as *wingless*, *blistered*, *delta,* and *cut*, resulting in mis-regulation of vein and margin cell fate. We found that Zelda binds near genes important for EGF, TGF-b, Notch, Dpp and Hh signaling, which could account for Zelda's role in vein cell fate. Inactivation of Zelda during regeneration also reduced the expression levels of *taranis* and *osa*, which we have previously shown act to ensure correct *engrailed* expression during regeneration, protecting posterior cell fate from disruption by regeneration signaling. Zelda was also bound near and required for expression of integrins, accounting for the wing blistering phenotype. Thus, this study reveals a key role for Zelda in regulating cell fate and patterning genes during the transition from regeneration back to normal development.

260 **Tissue regeneration following necrosis requires non-apoptotic caspase activity** Jacob W Klemm, Robin Harris School of Life Sciences, Arizona State University

Necrosis is a catastrophic type of tissue death that can occur in almost any tissue type and arise from several types of injury and inherited and congenital conditions. Characterized by the sudden loss of membrane integrity, necrotic cell death often spreads to adjacent, healthy cells, resulting in collective tissue death. A better understanding of how necrotic cells interact with the surrounding tissue is crucial to effectively treating necrotic wounds. However, a lack of accessible genetic models to study the interactions between necrotic and healthy cells has impaired progress in the field. To address this, we established a novel genetic ablation system to drive necrotic cell death in the *Drosophila* wing imaginal disc and study the tissue response that follows.

With this model, we quickly found that tissues are competent to regenerate from necrosis and this regenerative response is unique to necrotic ablation. Following damage, significant apoptosis is induced at a distance from the wound, which we call necrosis-induced apoptosis (NiA). Unlike typical damage-associated apoptosis, NiA cells are not regulated by the JNK pathway, a highly conserved regulator of both apoptosis and regeneration. However, NiA are necessary for regeneration; limiting NiA activity results in a reduced capacity to regenerate, as assayed by adult wing size. Thus, following necrosis, tissues respond by inducing NiA to promote regeneration.

We are currently investigating the precise mechanism by which NiA cells promote tissue repair and have found that NiA promote regenerative cell proliferation. EdU labeling reveals high levels of proliferation at the wound edge that is abolished when NiA activity is blocked via RNAi. However, NiA cells do not appear to secrete mitogens involved in apoptosis-induced proliferation (AiP), suggesting that NiA may promote proliferation via a different mechanism. Using a caspase sensitive GFP reporter to label NiA cells, we have found that NiA appear to persist in the disc proper throughout regeneration, suggesting that these cells may survive apoptotic signaling to directly repair the tissue. To test this hypothesis, we are designing caspase-based lineage trace experiments to track the fate of NiA cells and determine if NiA contribute to the regenerated wing tissue. As NiA represent a unique response to tissue necrosis, further characterization may inform the treatment of necrotic wounds in a clinical setting.

261 **Unscheduled endocycles impair growth of the *Drosophila* wing disc** Yi-Ting Huang, Lauren Lian Hesting, Brian R. Calvi Indiana University Bloomington Biology

How tissues grow and regenerate is incompletely understood. Some tissues grow through an increase in cell size (hypertrophy) by switching to a variant endocycle which cells grow and duplicate their genome through repeated G and S phases without division. Cells can also undergo an unscheduled switch to endocycles in response to aging, stress, and environmental inputs. We call these induced endoreplicating cells (iECs) to distinguish them from scheduled developmental endoreplicating cells (devECs). While iECs aid in wound healing and tumor suppression, they also contribute to cancer therapy resistance and tumor regrowth. Much remains unknown, however, about the regulation and growth of these unscheduled iECs and how they impact normal or pathological growth.

We have been using the *D. melanogaster* wing disc as an *in vivo* genetic model to evaluate how unscheduled iECs affect growth. To address whether iEC hypertrophic growth can replace cell division for tissue growth, we induced iECs in clones and also in different regions of the wing disc by using GAL4/UAS to inhibit Cyclin A. We found that iECs initially grew in cell size and accumulated total tissue mass at a rate proportional to mitotically-dividing controls, but then later iEC growth slowed and resulted in significant tissue undergrowth. Further analysis of endocycle dynamics revealed variation among different iECs, which arrested at different terminal ploidies. These iECs had DNA repair foci near heterochromatin suggestive of replication stress. Analysis of our RNA-Seq data from S2 iECs revealed an upregulation of numerous genes that are associated with

senescent cell cycle arrest. We found that these genes are also upregulated in wing disc iECs *in vivo*, suggesting that iEC growth is limited by a senescent-like arrest. These cells also had activated Jun N-Terminal Kinase (JNK) stress pathway. Inhibiting JNK activity revealed that JNK signaling mediates both senescent-like response and iEC growth arrest. We are currently evaluating how iECs influence proliferation of neighboring cells and the extent to which it resembles a wounding and regeneration response. Altogether, our results indicate that growth of unscheduled iECs is limited by a senescent-like arrest that has severe negative consequences for tissue growth. More broadly, our findings are providing clues to how iECs may paradoxically cause human tissue undergrowth or contribute to increased cell proliferation during tumorigenesis.

262 **Nutrient-dependent dedifferentiation in the midgut epithelium promotes adaptive growth in *Drosophila*** Yuichiro Nakajima[1], Hiroki Nagai[1], Luis Augusto Eijy Nagai[2], Sohei Tasaki[3], Ryuichio Nakato[2], Masayuki Miura[1] [1]Graduate School of Pharmaceutical Sciences, University of Tokyo, [2]Institute for Quantitative Biosciences, University of Tokyo, [3]Graduate School of Science, Hokkaido University

Post-developmental organ resizing improves organismal fitness under constantly changing nutrient environments. Although stem cell abundance is a fundamental determinant of adaptive resizing, our understanding of its underlying mechanisms remains primarily limited to the regulation of stem cell division. In mammals, cellular plasticity, or dedifferentiation, has been identified in multiple tissues, especially in the intestinal epithelium where both absorptive and secretory lineages undergo dedifferentiation into intestinal stem cells (ISCs) upon severe injury or during inflammatory tumorigenesis. However, it is remains largely unclear whether cell fates are plastic under physiological conditions or as the result of naturally occurring perturbations. Here we demonstrate that nutrient fluctuation induces dedifferentiation of enteroendocrine cells (EEs) in the *Drosophila* adult midgut to drive adaptive intestinal growth. From lineage tracing and single-cell RNA-sequencing, we identify a subpopulation of EEs, or AstC-positive EEs that convert into functional ISCs in response to dietary glucose and amino acids by activating the JAK-STAT pathway. We further show that EE-derived ISCs are multipotent but preferentially generate new enterocytes, leading to a rapid increase in gut size. Genetic ablation of EE-derived ISCs severely impairs ISC expansion and midgut growth despite the retention of resident ISCs, and *in silico* modeling further indicates that EE dedifferentiation enables efficient increase in the midgut cell number while maintaining epithelial cell composition. Our findings thus uncover a physiologically-induced dedifferentiation that ensures ISC expansion during adaptive organ growth in concert with nutrient conditions.

263 **Two sequential gene expression programs bridged by cell division support long-distance collective cell migration** Jingjing Sun[1], Aswini Babu[1], Frank Macabenta[2], Ayse Damla Durmaz[1], Angelike Stathopoulos[1] [1]California Institute of Technology, [2]California State University Monterey Bay

The precise assembly of tissues and organs relies on spatiotemporal regulation of gene expression within migrating collectives to coordinate cell behavior. In Drosophila embryos, the midgut musculature is formed through collective migration of caudal visceral mesoderm (CVM) cells, but how gene expression changes as cells migrate is not well understood. Here, we focused on ten genes expressed in the CVM and cis-regulatory sequences controlling their expression. While some genes are continuously expressed, others are expressed only early or late during migration. Late expression relates to cell cycle progression, as driving string/Cdc25 causes earlier division of CVM cells and precocious expression of late genes. In particular, we found that cell cycle effector transcription factor E2f1 is a required input for late gene CG5080. Furthermore, while late genes are broadly expressed in all CVM cells, early gene transcripts are polarized to anterior or posterior ends of the migrating collective. We show this polarization requires transcription factors Snail, Zfh1, and Dorsocross. Collectively, these results identify two sequential gene expression programs bridged by cell division that support long-distance directional migration of CVM cells.

264 **Regulation of the Competency to Amplify Intermediate Progenitor generation during Neurogenesis** Cyrina M Ostgaard[1], Arjun Rajan[2], Cheng-Yu M Lee[2] [1]Cell and Developmental Biology, University of Michigan, [2]University of Michigan

Intermediate progenitors (IPs) are neural stem cell progeny that undergo limited proliferation to increase neuron production. Subsets of these cells likely function as transit-amplifying cells to increase IP generation per asymmetric stem cell division. How this competency is instilled in stem cells is unknown largely due to the inability to define functionally relevant enhancers. I used fly larval brain neural stem cells (neuroblasts; NBs) to investigate regulation of the competency to amplify IP generation because cell types in NB lineages are well characterized. A type I NB asymmetrically divides to generate a ganglion mother cell (GMC) that produces two neurons. By contrast, a type II NB generates an intermediate neural progenitor (INP) that amplifies GMC generation increasing neuron production 6-fold or more per asymmetric division of a type II NB compared to a type I NB. We combined cell type-specific chromatin accessibility, occupancy by functionally relevant transcription factors and histone marks to define 4332 neurogenic enhancers in NBs. I confirmed the sufficiency of dozens of these candidate enhancers to drive reporter expression in larval brain NBs. Our data suggest that Sp/KLF family transcription factor Buttonhead (Btd; Sp8 in vertebrates) promotes NB competency to generate INPs while achaete-scute family transcription factor Asense (Ase; Ascl1 in

vertebrates) limits this competency. By correlating Btd-bound neurogenic enhancers to transcripts uniquely enriched in type II NBs and INPs, we identified 19 transcription factors including Bi (Tbr-2 in vertebrates) that likely function downstream of Btd to promote INP generation. We also identified 24 transcription factors that likely function downstream of Ase to inhibit INP generation based on their neurogenic enhancers containing a human Ascl1-binding motif and their transcripts enriched in type I NBs or INPs. I will combine loss- and gain-of-function approaches to validate the roles of these candidate regulators of the competency to generate INPs in NBs. I am also testing whether the Ase regulatory network functions as a barrier to reprogramming type I NBs to generate INPs following Btd mis-expression. Strategies that increase or decrease the generation of differentiated cells on-demand by manipulating stem cell competency to transiently amplify IPs could increase the efficiency of tissue regeneration and provide novel therapeutic targets for treating certain childhood tumors.

265 **Stress-responsive keratins regulate innate immunity in inflamed skin** Erez Cohen[1], Yang Xu[2], Craig N Johnson[1], Kaylee Steen[1], Johann E Gudjonsson[3], Carol A Parent[2,4,5], Pierre A Coulombe[1,3,5] [1]Cell and Developmental Biology, University of Michigan Medical School, [2]Pharmacology, University of Michigan Medical School, [3]Dermatology, University of Michigan Medical School, [4]Life Sciences Institute, University of Michigan, [5]Rogel Cancer Center, University of Michigan

A small group of keratin intermediate filament genes, the type II *KRT6* (protein K6A-C) paralogs and the type I *KRT16* (K16) and *KRT17* (K17), are robustly induced in surface epithelial keratinocytes in response to environmental stressors and in inflammatory skin diseases, including psoriasis, atopic dermatitis, and others. Mutations in these keratins cause Pachyonychia Congenita (PC), a rare genetic disorder showing striking anomalies in differentiation and homeostasis in ectoderm-derived epithelial appendages and glabrous skin. Accumulating evidence has highlighted multiple roles for keratins in regulating cellular signaling, yet the role of stress-responsive keratins in differentiation and inflammation remains ill-defined. By combining single cell transcriptomic data from human inflammatory skin diseases, *in-vivo* mouse models of single keratin deficiencies along with *ex-vivo* keratinocyte cell culture models, we identified stress-responsive keratins as potent regulators of innate immune responses. Further, and despite the high sequence homology between them, we show that K16 and K17 associate with distinct molecular pathways and exert opposing impacts on immune responses.

Both K16 and K17 are induced in keratinocytes within hours after exposure of mouse skin to stressors. Additional exposure to topical stresses within 24 hours after their induction results in a transient amplification of the neutrophil influx into the skin. Studies in *Krt17* null mice and *KRT17* null human keratinocyte cultures show that this response requires a K17-dependent sustainment of Protein Kinase C a activity and consequent release of neutrophil chemoattractants by stressed keratinocytes. In stark contrast, parallel studies involving *Krt16* null mice and *KRT16* null human keratinocyte cultures suggest that K16 functions to dampen innate immune activation by attenuating cytokine release in response to stressors. Preliminary evidence further suggest that K16 is both co-regulated and physically interacts with activators of type I interferon response, an established signaling pathway activated in stress-responsive keratinocytes. These data introduce a new paradigm uncovering the complexity of the interplay between stress-responsive keratins and innate immunity in skin, with significant implications for chronic inflammatory skin diseases and the rare genetic disease Pachyonychia Congenita.

266 **Evidence that Orthologous Loci Containing R-spondin 2 Regulate Respiratory Responses to Air Pollution in Mice and Humans** Samir Kelada[1], Adelaide Tovar[2], Gregory J Smith[1], Heather P. Wells[1], Neil E Alexis[1], David P. Peden[1] [1]University of North Carolina, [2]University of Michigan

Respiratory responses to air pollution exposure are complex traits, but few loci that contribute to variation in response have been identified and/or replicated in either humans or mice. We focus here on responses to ozone (O_3), an ambient air pollutant common in urban areas. Laboratory studies have shown that acute O_3 exposure causes airway inflammation and epithelial injury that varies widely across humans and inbred strains of mice. Additionally, epidemiologic studies have shown that ambient O_3 exposure is associated with the onset and/or exacerbation of both asthma and COPD. We sought to identify novel genetic loci that regulate responses to O_3 using the Collaborative Cross (CC) mouse genetics reference population, and then test whether orthologous loci also influence responses in humans. To that end, we conducted a QTL mapping experiment utilizing 56 CC strains, quantifying O_3-induced inflammation and injury phenotypes. We identified a suggestive QTL for lung injury on Chr 10 (peak = 26.2 Mb; confidence interval [CI]: 24.6–43.6 Mb)) and a genome-wide significant QTL on Chr 15 (peak = 47.1 Mb; CI: 40.2–54.9 Mb). We subsequently narrowed the Chr 15 QTL region to 41-45 Mb, a region containing 10 protein coding genes, through additional analysis of the QTL allele effects and CC founder strain haplotypes. In a subsequent experiment with CC mice, we replicated the QTL effect and evaluated the expression of the 10 candidate genes. We found that the expression of one candidate gene, *Rspo2,* paralleled lung injury over time after O_3 exposure, with lower expression linked to higher lung injury. Using a neutralizing antibody approach, we depleted RSPO2 in mice and found that this led to heightened O_3-induced lung injury and inflammation. Serendipitously, an epidemiologic study reported a genotype-by-air pollution exposure interaction in COPD with rs10086579, a common genetic variant near human *RSPO2*. We then genotyped 75 human volunteers who previously underwent an acute O_3 exposure in an environmental chamber and found a highly suggestive

association between rs10086579 and O_3-induced airway inflammation. Collectively, these results suggest that genetic variation in orthologous regions of the mouse and human genomes are associated with response to O_3 and that these genotype-by-exposure interactions may alter risk of COPD.

267 **A European effort for therapeutic gene editing in Rett syndrome: from organoids to mouse models** Tania Sorg[1], Susanna Croci[2], Andrea Rosetti[3], Angel Edo[4], Caterina Lo Rizzo[5], Sergio Daga[2], Maria Antonietta Mencarelli[5], Roberto Canitano[6], Marina Sica[7], Francesco Molinaro[7], Lino Nobili[8], Giulia Prato[9], Giulia Nobile[9], Silvio Boeri[9], Aglaia Vignoli[10], Maria Paola Canevini[11], Ilaria Vigano[12], Mario Chiariello[13], Miguel Chillon[14], Julia Ladewig[15], Yann Herault[1], Alessandra Renieri[2], Ilaria Meloni[2] [1]PHENOMIN - ICS, [2]Medical Genetics, University of Siena, [3]Central Institute of Mental Health (ZI), [4]Vall d'Hebron Research Institute, [5]Genetica Medica, Azienda Ospedaliero-Universitaria Senese, [6]Division of Child and Adolescent Neuropsychiatry, University Hospital of Siena, [7]Pediatric Surgery, Department of Women and Children, S. Maria alle Scotte Hospital, University of Siena, [8]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, [9]Child Neuropsychiatric Unit, Instituto G. Gaslini, [10]Health Sciences Department University of Milano, [11]Health Sciences Department, University of Milano, [12]Epilepsy Center, ASST Santi Paolo Carlo, [13]Core Research Laboratory, ISPRO Institute for Cancer Research, Prevention and Clinical Network, [14]Vall d'Hebron Research Institute, Catalan Institution for Research and Advanced Studies, [15]Central Institute of Mental Health, Heidelberg University/Medical Faculty Mannheim

Rett syndrome is a rare neurodevelopmental disorder and the second most common cause of intellectual disability in women after Down syndrome. Classic form of the disease is caused by *MECP2* mutations. Reactivation of *Mecp2* in symptomatic KO mice can revert disease phenotypes, suggesting that RTT is not irreversible. Gene editing represents an extremely promising therapeutic approach, since it allows maintaining the endogenous regulatory framework. The approach recently made its way to clinical trial for Leber Congenital Amaurosis (NCT03872479), demonstrating its translational potential. To validate its applicability to the 4 most common *MECP2* hotspot variants, we established an international consortium that was funded from the EU in 2021. We engineered a CRISPR/Cas9 gene editing toolkit composed of a dual plasmid system specific for each *MECP2* mutation. We collected RTT samples and reprogrammed iPSCs from 2 patients for each variant to generate brain organoids, 3D structures which closely mimic early human brain development *in vitro*. RTT organoids have been generated for selected mutations and undergo a detailed characterization. In RTT 3D models we are also validating the infection efficiency of the available chimeric AAV7m8 virus that has been chosen for the *in vitro* experiments.

In addition, NGS analysis in RTT fibroblasts obtained from *MECP2* patients demonstrated a high editing efficiency, in particular up to 90% and 72% of HDR for R306C and for R168X variants respectively. We tested also AAV7m8 in iPSC-derived neurons with T158M and evaluated HDR efficiency. However, these viruses are still far from optimal since a significant portion spreads to peripheral organs reducing the efficiency of the approach and increasing the risk of toxic effects. For this reason, new chimeric serotypes with higher specificity and selectivity have been designed and are currently under characterization.

Finally, mouse model colonies harbouring 3 variants have been generated to further test the approach *in vivo*. A behavioural phenotyping pipeline has been defined and allowed us to characterize the clinical features in our conditions. One of these variants will be used in combination with the most promising AAV vector to evaluate the rescue *in vivo*.

Our work will represent a real opportunity for the application of gene therapy for RTT, thus providing the first real possibility for an RTT therapy.

268 **Humanization of the mouse telomerase reverse transcriptase gene reset telomeres to human length** Fan Zhang, De Cheng, Suwen Wang, Jiyue Zhu College of Pharmacy and Pharmaceutical Sciences, Washington State University

Telomeres shorten with each cell division and eventually cause replicative senescence, serving as a biomarker of human aging. Mice, however, have excessively long telomeres and exhibit widespread telomerase activity, which constrains their utility as models for human diseases and longevity. To overcome this challenge, we genetically engineered a humanized version of the mouse telomerase reverse transcriptase gene, *Tert* $^{(tm1\ (TERT)Jzhu)}$ (*hmTert*) in embryonic stem cells, by replacing the 5' intergenic region, introns 2 and 6 of the mouse *Tert* gene with human counterparts that contained multiple known regulatory elements of the human *TERT* gene. Mice with a germline *hmTert* gene were generated and bred in the C57BL/6J genetic background. The *hmTert* gene, encoding the unaltered mouse TERT protein, was repressed in most adult tissues and only expressed in gonads and a limited subset of immune cells, mimicking the regulation of the human *TERT* gene. The *hmTert* gene rescued telomere dysfunction in late generations of *mTert*-knockout mice. Through successive intercrosses of B6;129S6-*Tert*$^{tm1(TERT)Jzhu}$ mice, telomere length decreased progressively and eventually stabilized at an average length below 10 kb. The *Tert*$^{h/h}$ mice acquired an average 10-12 kb telomere length resembling that of humans, in contrast to the over 50-kb average telomere length found in C57BL6J mice. Despite having very short telomeres and no telomerase in most adult tissues, these mice

displayed normal body weight and maintained cellular homeostasis in high-proliferative tissues, including testes, intestine, and bone marrow. Our data demonstrate that humanizing the regulation of the mouse*Tert* gene resets mouse telomeres to human lengths. This transformation provides mice with humanized telomere homeostasis and a promising model to explore essential aspects of human aging and cancer.

269 **A missense mutation in *Birc6* causes dwarfism in mice** Julie Ruston[1], Zachary Klugman[1,2], Danilo J Shevkoplyas[1], Monica J Justice[1,2] [1]Genetics and Genome Biology, Hospital for Sick Children, [2]Molecular Genetics, The University of Toronto

Our lab identified a mouse line with a small size phenotype in a dominant N-ethyl-N-nitrosourea (ENU) mutagenesis screen for suppressors of the phenotype in methylCpG binding protein 2 (*Mecp2*) mice. Sequence analysis found a missense mutation in a highly conserved arginine in Baculoviral inhibitor of apoptosis repeat-containing 6 (*Birc6)*, a member of the inhibitor of apoptosis protein (IAP) family. BIRC6 plays a role in inhibiting apoptosis, but has been implicated in additional molecular pathways including autophagy, cell cycle regulation and the DNA damage response. Homozygotes for a null allele die at birth due to placental failure, yet heterozygotes for a null allele are normal in size. The causative nature of the lesion was confirmed using CRISPR/Cas genome editing to engineer an identical allele, which also has a dominant small size phenotype. *Birc6*$^{Sum20\text{-}Jus}$/+ mice are smaller than littermates from mid-gestation through adulthood. At three months, the mice are approximately 50% of the size of their wild type littermates, exhibiting proportional dwarfism with pathological changes in bone, thyroid and pituitary. IGF1, but not growth hormone (GH) is decreased, and the mice have primary hypothyroidism (high TSH, normal T4). Tandem mass tag (TMT) spectrometry quantification of proteins in the brain links BIRC6 mutation to perturbations in cell cycle and metabolic pathways. Together, our data suggest that mutations in BIRC6 cause a dominant small stature phenotype due to signalling anomalies that perturb the endocrine pathway. An association between *Birc6* and dwarfism has not been previously reported; therefore, this work identifies a new model of proportional dwarfism and a new function for BIRC6.

270 **The host-transposase fusion *THAP7* is a transcription factor implicated in vertebrate development and human intellectual disability** Rachel L Cosby[1], Jennifer Sinclair[1], Catrina Rateb[1], Steven Gay[1], Lisa Kratz[2], Jennifer Panlilio[1], Alban Ziegler[3], Fowzan S Alkuraya[4], Harold A Burgess[1], Todd S Macfarlan[1] [1]National Institute of Child Health and Human Development, [2]Kennedy Krieger Institute, [3]Centre Hospitalier Universitaire d'Angers, [4]KFSHRC

Transcription factors (TF) and their networks can evolve via fusion between host- and DNA transposase-derived protein domains. Several host-transposase fusions have been implicated in vertebrate development and developmental disorders, but the function of most remain unknown. We identified homozygous and compound heterozygous missense variants in human *THAP7*, a TF derived from a *P*-element-like transposase fusion, which segregate with intellectual disability in two families, suggesting *THAP7* regulates neurodevelopment. To determine THAP7 function in development, we generated THAP7 mutant mice and zebrafish using CRISPR technology. We demonstrate that *Thap7* null mice are viable, with males displaying subtle but significant impairments in working memory and contextual learning, while females display deficits in spatial and contextual learning. Thus, *Thap7* null mice partially recapitulate the human phenotype.

In contrast, *thap7* null zebrafish died shortly after juvenile stages and were significantly smaller than their WT siblings. Using RNAseq and ChIPseq, we found that Thap7 binds the promoters of the glutaryl-CoA dehydrogenase a and b (*gcdha/b*) genes, whose expression is silenced in *thap7*$^{-/-}$ larvae. Human GCDH deficiency causes glutaric acidemia type I (GA1), where glutaric acid (GA) accumulation leads to striatal neuron degeneration and death. We hypothesized that downregulation of *gcdha/b* leads to GA accumulation and subsequent growth impairment and death of *thap7*$^{-/-}$ fish. Using mass spectrometry, we found that *thap7*$^{-/-}$ larvae have ~7 fold higher GA and detectable 3-hydroxy glutaric acid (3-OH-GA) compared to their sibs, suggesting that *thap7*$^{-/-}$ fish die due to GA1-like symptoms.

The disparate phenotypes between fish and mammals suggested that THAP7's function may have diverged in vertebrates. We performed THAP7 ChIPseq in juvenile mouse brains and mouse (mESCs, 3T3, Neuro2a, and GC1), human (293T, K562, HepG2), and zebrafish (ZFL) cell lines to identify THAP7 target genes. We found that while THAP7 binds a significant number of gene promoters in two or more species (n=1679, ~50%), it also binds some promoters in only one cell type or species. Conserved THAP7 targets are enriched for fundamental housekeeping processes including RNA processing and cell proliferation, whereas zebrafish specific THAP7 targets are enriched for developmental processes such as organ development and morphogenesis (Biological Process GO), consistent with the more severe developmental phenotype seen in fish. Our results suggest that, while THAP7's molecular function as a TF is preserved, its biological function has diverged between fish and mammals. Our research underscores THAP7's role in vertebrate development and human intellectual disability, and elaborates on the origin and function of host-transposase fusion genes.

271 **Defects in maternal LSD1/KDM1A reprogramming may contribute to phenotypes observed in human neurodevelopmental disorders** Carrie Eilleen Falkenberry[1], Monica Reeves[2], Rhea Rastogi[2], Alyssa Scott[2], David John Katz[2] [1]Cell Biology at Emory University, Emory University, [2]Cell Biology, Emory University

The lysine demethylase 1A (LSD1/KDM1A) is an epigenetic reprogramming enzyme that removes H3K4me1/2 and is required maternally for proper development in *C. elegans* and mice. Complete knockout of maternal KDM1A in mice results in embryonic arrest at the 2-cell stage due to failure to shut off maternal genes during the maternal to zygotic transition. This finding led us to consider the possible roles of maternal KDM1A in inherited disease. To study this, we developed a hypomorphic *Kdma1a* allele (M448V) in mice that decreases the ability of KDM1A to bind to RCOR1/2(CoREST), resulting in inefficient demethylation. When KDM1A reprogramming is partially compromised only maternally, progeny show a high rate of perinatal lethality, developmental delay, craniofacial abnormalities and abnormal behavior. Three of these phenotypes, developmental delay, craniofacial abnormalities and abnormal behavior, are also observed in KDM1A patients and in patients with the related Kabuki Syndrome. This raises the possibility that maternal defects could contribute to these diseases. To take an initial look at what changes might be inherited when KDM1A is hypomorphic maternally, we also performed RNAseq in progeny at embryonic day 13.5 (e13.5). Despite the fact that these embryos showed no obvious phenotypes, the overall gene expression changes can be distinguished by maternal genotype. This suggests that failure to completely reprogram KDM1A maternally in the oocyte can result in heritable genes expression changes in the progeny. Intriguingly, using gene ontology analysis, we observe that mammalian phenotypes associated with the genes that are significantly upregulated in our RNAseq analysis include decreased body weight/postnatal growth retardation and preweaning lethality, both of which we observe later in development in the progeny of mothers with maternally compromised KDM1A reprogramming. This hints that genes that may be causing the perinatal lethality and developmental delay may already be starting to be misexpressed at e13.5 272 **Chance, individuality, and phenotypic variation in health and disease** Joseph Nadeau[1], Andrew Pospisilik[2] [1]Maine Medical Center, [2]Van Andel Institute

What if our ideas about phenotypic variation overlook a primary determinant? Fisher's century-old variance model, Phenotype V_P = genetics V_G + environment V_E + error V_e, guides many areas of biological research. But substantial phenotypic variation is not represented in this model. On average 50% of variability is neither genetic nor environmental. We propose that considerable non-G and non-E variation emerges during development, arising from stochastic noise that triggers probabilistic epigenetic changes that once established are stable and deterministically propagated throughout life. These epigenetic changes lead to wide variation in phenotypic outcomes across many traits, in many species, and in health and disease. Individuality emerges from this interplay between genes, environment and chance. A deep understanding of organismal biology and the goals of Precision Medicine depend on discovering the mechanistic origins and systems properties of this surprisingly pervasive and strong but generally neglected dimension of phenotypic variation. I will share evidence and arguments about chance as a primary driver of non-G non-E phenotypic variation, propose an *'accidental individuality'* model, and suggest a phenotype-driven strategy to improve diagnosis and treatments for disease involving a virtuous cycle of discovery, experiments and translation between model organisms and humans.

273 **The nonsense-mediated mRNA decay pathway degrades regulatory non-coding RNAs** David J Young, Nicholas R Guydosh Laboratory of Biochemistry and Genetics, NIDDK/NIH

The nonsense-mediated mRNA decay (NMD) pathway targets mRNAs undergoing premature translation termination for degradation, including nonsense alleles associated with human disease. One potential therapeutic approach for such cases involves stabilization of the mRNA via pharmaceutical suppression of NMD and induction of stop codon readthrough. It is therefore important to understand the natural role of NMD in the cell. RNA-seq of yeast lacking NMD surprisingly revealed that >80% of genes targeted by NMD appear to lack obvious premature termination codons (PTCs). One possible explanation for this targeting is that translation of cryptic open reading frames (ORFs) results in widespread premature termination events. To test this hypothesis, we developed a combined approach using RNA-seq to identify transcript isoforms targeted by NMD and a novel 40S ribosome profiling strategy to find the boundaries of cryptic ORFs by using peaks on start and stop codons. We identified novel translation events on approximately three quarters of the targets that initially appeared to lack PTCs. Interestingly, most of these cryptic events occur on non-coding RNA (ncRNA) transcript isoforms. Many of them appear to have regulatory roles in two-promoter systems and share properties with long un-decoded transcript isoforms (LUTIs). Transcription of LUTIs has been shown to repress the canonical gene promoter through repressive chromatin marks. We found these LUTI-like transcripts generally encode multiple 5' upstream ORFs (uORFs) that often trigger NMD. These LUTI-like transcripts have roles in many metabolic pathways, for example nitrogen sensing where they are poised to regulate expression of *DAL5, DAL7*, and other genes. Elimination of uORFs in the *DAL5* and *DAL7* LUTI-like transcripts resulted in their stabilization, thus confirming that termination events at 5' uORFs sensitize these transcripts to NMD. We also inserted a sequence that induces transcription termination into the *DAL5* LUTI-like transcript and observed derepression of a short *DAL5* transcript that originates from a downstream promoter. This confirms that transcription from the *DAL5* LUTI-like promoter inhibits the downstream canonical promoter. The ncRNAs produced from these LUTI-like promoters are a novel class of NMD substrates. Furthermore, NMD is critical for removing these repressive regulatory mRNAs from the cytoplasm of the cell, potentially to prevent unproductive translation and the synthesis of toxic peptides.

274 **Understanding the mechanisms controlling deposition and interplay of co-transcriptional nucleosome modifications** Tasniem Fetian, Brendan M McShane, Karen M Arndt Biological sciences, University of Pittsburgh

The wrapping of eukaryotic DNA around histones to form nucleosomes allows efficient DNA packaging but presents a barrier to proteins that perform DNA-templated transactions. Multiple regulatory mechanisms have therefore emerged to modulate nucleosomal properties and DNA accessibility. Monoubiquitylation of H2B (H2Bub) on K123 in budding yeast (K120 in humans) is a dynamic co-transcriptional histone modification that modulates chromatin on structural and functional levels. In yeast and higher eukaryotes, the Paf1C transcription elongation factor is required for H2Bub and facilitates its coupling to transcription. A small domain within the Rtf1 subunit of Paf1C, named the Histone Modification Domain (HMD), directly interacts with the multi-functional ubiquitin conjugase Rad6 leading to H2Bub stimulation both in vivo and in vitro. To understand how Rad6 is recruited to its histone substrate, we employed a multi-faceted approach to identify the site of HMD interaction within Rad6. By combining genetic, biochemical, and genomic approaches, we characterized separation-of-function mutations in *S. cerevisiae RAD6* that disrupt the Rad6–HMD interaction and hence H2Bub, while other functions of Rad6 are largely unchanged. Mutating either side of the proposed Rad6–HMD interface results in virtually indistinguishable transcriptome profiles as measured by RNA-seq. Our results promote a model in which a highly specific Rad6-HMD interface selectively targets Rad6 to its nucleosomal substrate during transcription. While the molecular events that couple H2Bub to transcription are now coming into focus, the direct contributions of this conserved modification to transcription and its coordination with other epigenetic modifications remain to be fully elucidated. Previous studies revealed a synthetic lethal relationship between H2Bub and H2A.Z, the H2A histone variant that is enriched at the +1 nucleosome of genes. However, it is unclear how these two epigenetic modifications are coordinated and why cells lacking one of the two are so dependent on the other for viability. Our ongoing genetic and genomic experiments are directed towards investigating the impact on the transcriptome upon the concurrent loss of H2A.Z and H2Bub and pinpointing mechanisms that are responsible for the synthetic lethality.

275 **Insights into yFACT-gene interactions and the mechanisms underlying a human developmental disorder through analyses of histone mutants in yeast** Andrea A Duina, Alex Pablo-Kaiser, McKenzie G Tucker, Grace A Turner, Elijah G Dilday, Avery G Olmstead, Caroline Tackett, Rose Johnson, Shelby Worsham, Joey Beard, Reece Forrest, Lillian Francis, Will Griffin, Agustin Kalinowski, Jackson Parks Biology Department, Hendrix College

In previous work in yeast, we identified a region of the nucleosome whose integrity is required for proper interactions between the histone chaperone complex yFACT and genes. More specifically, mutations within this region, which we refer to as ISGI (Influences Spt16-Gene Interactions), cause a shift in the distribution of yFACT toward the 3' ends of transcribed genes, an effect that we have attributed to impaired yFACT dissociation from genes following the transcription process. Interestingly, an arginine substitution at the ISGI residue H3-L61 within the human histone H3.3 protein (H3.3 L61R) has recently been shown to be associated with a novel neurodevelopmental condition in humans called Bryant-Li-Bhoj syndrome, raising the possibility that our work in yeast may provide insights into this condition.

In this presentation, I will first describe preliminary results from ChIP-seq experiments that suggest that ISGI mutants interfere with proper yFACT dissociation from genes at the genome-wide level in yeast. I will then present data in support of the notion that the defects seen in yFACT-gene dissociation due to expression of H3-L61R in yeast may be informative in the understanding of the molecular defects underlying the Bryant-Li-Bhoj syndrome in human patients. Finally, I will discuss ongoing experiments to assess the functional defects caused by additional amino acid substitutions identified across the human H3.3 protein that are also associated with the Bryant-Li-Bhoj syndrome using yeast cells as model system. Overall, our work is providing further insights into the impact of ISGI mutants on yFACT-gene interactions across the yeast genome and into the molecular defects caused by histone mutants that have been implicated in a neurodevelopmental condition in humans.

276 **Regulation of heterochromatin formation and mRNA export via potential targets of Dsk1 and Kic1 kinases** Aditi Vyas[1], Anna Freitas[1], Zachary Ralston[1], Margaret Nurimba[1], Xiaojing Yang[2], Chao Tang[2], Bashar Alhoch[1], Peter Zang[1], Camille Ylagan[1], Alanna Sugarman[1], Zhaohua Tang[1] [1]W. M. Keck Science Department, Claremont McKenna College, Scripps College and Pitzer College, [2]Center for Quantitative Biology, Peking University

Dsk1 and Kic1/Lkh1 are LAMMER-related kinases and while members of this kinase family have been previously identified as important regulators of cellular function with roles in mitotic cycle, differentiation and cellular development, the complex biological functions and molecular targets through which these kinases regulate cellular pathways remain unclear. Our previous research suggests that Dsk1 and Kic1 kinases play a role in heterochromatin formation and nuclear export of mRNA. To investigate possible functions of the kinases, we performed a genome-level epistasis assay. Rresults from that screening provide strong evidence for genetic interaction between Dsk1 kinase and components of the DASH complex - a complex of kinetochore associated proteins. This data, combined with the results from a functional heterochromatin silencing assay, and the similarity in nuclear localization between Kic1 and Swi6/HP1 implicate the role of both, Dsk1 and Kic1, in centromere

heterochromatinization. Additionally, GFP-tagged Swi6 protein molecules appear mildly delocalized to the cytoplasm in the kinase-deletion strains. Immunofluorescence analysis in WT cells confirms the co-localization of GFP-Kic1 with Swi6 in the nucleus, supporting the notion of a functional relationship between Kic1 and Swi6. Another line of experiments uncovered a role of the Dsk1 and Kic1 kinases in nuclear export of mRNA, independent from the known role of the kinases in mRNA splicing. Results from a biochemical investigation also identified the Poly(A) binding protein (Pabp) in a physical complex with Dsk1. Using Pabp-GFP tagged strains we have been able to confirm that while the WT strains exhibit a ubiquitous Pabp protein distribution through the cellular cytoplasm and nucleus, the kinase deletion strains display a higher nuclear retention pattern of Pabp molecules. Consistently, results from FISH assays show some degree of nuclear retention and co-localization of the global mRNA population with the Pabp protein molecules in the kinase-deletion strains. Moreover, western blot analysis reveals a reduction of total Pabp protein in the *dsk1-* and *kic1-* deletion strains when compared to WT. Our results, taken together, indicate that Dsk1 and Kic1 may exert their effect on centromeric heterochromatinization via Swi6/HP1 protein and affect mRNA export through modulating Pabp protein stability and cellular localization.

277 **Allelic variation in codon bias and mRNA folding stability act on transcript abundances, translation, and protein abundances via both shared and distinct mechanisms in yeast** Daniel A Pollard[1], Anastacia Wienecke[2], Maggie Barry[3], Nadine Tietz[4] [1]Biology, Western Washington University, [2]University of North Carolina, [3]University of Oregon, [4]Western Washington University

Heritable variation in protein abundance is a major driver of trait variation and yet the mechanisms by which polymorphisms in gene transcripts act on transcription, mRNA stability, translation, and protein stability are not well resolved. Properties of transcripts such as usage of codons with more abundant cognate tRNAs (codon bias) and mRNA folding strength (mF) are strongly implicated factors, and yet the specific mechanisms of their actions and how they interact with each other, and other properties of transcripts, are open questions for the field. Using RNAseq and mass spec data for 22 Saccharomyces cerevisiae isolates, we examined the association of polymorphic transcript properties with transcript abundance (TA), protein abundance (PA), and their ratio PA/TA for more than a thousand genes with linear mixed-effect models. We found that allelic variation in codon bias is positively associated with logTA, log(PA/TA), and logPA, consistent with slow translational elongation destabilizing transcripts. However, allelic variation in mF is negatively associated with logTA but positively associated with log(PA/TA) and logPA, suggesting structure may destabilize transcripts while also enhancing either translation or protein stability through unknown mechanisms. Despite the negative association between mF and logTA, we found that the interaction between codon bias and mF is positively associated with logTA, log(PA/TA), and logPA, suggesting a joint mechanism between codon bias and mF and a duel role for mF on TA. We next asked if the location in the transcript for a polymorphic codon or a polymorphic stem loop structure alters its effects. Codons throughout the CDS acted on logTA but only codons in domain encoding regions and near the 3' end of the CDS acted on log(PA/TA) and logPA, implying distinct mechanisms for codon bias acting on transcript stability and translation elongation. mF across the CDS consistently acted on logTA, log(PA/TA), and logPA, while mF just before the start codon impacted logTA and mF in 3' UTRs impacted log(PA/TA) and logPA, further supporting both shared and distinct mechanisms for mF acting at the transcript and protein levels. Our results present the most comprehensive characterization to date of how polymorphisms in transcripts influence protein expression.

278 **Modularizing the budding yeast transcription factor repertoire** Daniel T. Lusk, Zachary Krieger, Alessandro L.V. Coradini, Yiwei He, Chenghao Du, Cara B Hull, Nicolette E. Romo-Zelada, Oscar M. Aparicio, Ian M. Ehrenreich Department of Biological Sciences, USC

Natural genomes are disorganized, with functionally related genes typically spread across many chromosomes. This disorganization can make it difficult to study how sets of genes collectively give rise to cellular life and its diversity. Synthetic genomics approaches can be used to reorganize functionally related genes into modules, thereby producing organisms with simplified genomes that facilitate basic research and biotechnology. Such modularization is challenging because it requires building a neochromosome containing a set of functionally related genes and deleting the endogenous copies of these genes from their native loci. As a proof-of-principle for how to modularize many functionally related genes, we are producing a strain of the budding yeast Saccharomyces cerevisiae with nearly all ~200 RNA polymerase II-interacting transcription factors relocated to a synthetic neochromosome. This involves de novo synthesis of the transcription factor neochromosome and highly multiplexed deletion of endogenous transcription factor copies by CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a method we recently developed to build synthetic chromosomes from natural DNA. I will present the prototyping of our modularization strategy with Chromosome XVI, a roughly megabase sized chromosome containing ~20 transcription factors. I will also describe how our approach will be extended to the rest of the genome.

279 **The fourth zinc finger is a negative regulator of yeast transcription factor Fzf1** Ying Du, Wei Xiao Biochemistry, Microbiology and Immunology, University of Saskatchewan

A *Saccharomyces cerevisiae* transcription factor Fzf1 contains five Cys_2His_2 zinc finger domains and regulates at least five genes in response to diverse chemical stresses. *DDI2* and *DDI3* are duplicated genes that encode cyanamide hydratases and are induced by cyanamide; *SSU1* encodes a plasma membrane protein to efflux and reduces sulfite toxicity; *YHB1* encodes a dioxygenase and is induced by nitric oxide, while *YNR064c* encodes an epoxide hydrolase. Fzf1 coordinately regulates these genes through binding to a promoter consensus sequence CS2 using its N-terminal three zinc fingers (ZF1-3), while how ZF4 and ZF5 function remains unknown. Interestingly, amino acid substitutions in ZF4 including C157S, C162S and H180D have been reported to confer either sulfite resistance or increase basal-level *YHB1*expression, suggesting that Fzf1-ZF4 serves as a negative regulator. To test this hypothesis, we created yeast strains with these ZF4 point mutations and conducted cell survival and qRT-PCR assays to assess sodium sulfite resistance and Fzf1 downstream gene expression. These *fzf1-ZF4* mutants exhibited increased resistance to sodium sulfite compared to wildtype and *fzf1Δ* cells, and this resistance is dominant over wildtype *FZF1*. All *fzf1-ZF4* mutants displayed increased basal-level expression of *FZF1* downstream genes, and the degree of sodium sulfite resistance was highly correlated to *SSU1* transcript levels. A ZF4 international deletion mutant *fzf1-ΔZF4* behaved similarly as *fzf1-ZF4* point mutations, while Fzf1-ZF1-3 alone does not appear to support sodium sulfite resistance or its downstream gene expression, indicating that ZF5 plays a role in maintaining Fzf1 regulatory functions. In an EMSA assay, purified Fzf1 and Fzf1-ΔZF4 displayed comparable affinities for the target CS2 sequences, indicating that in the absence of relevant chemical stress, ZF4 inhibits Fzf1 activation instead of affecting its binding to the target CS2s.

280 **The Sex of the Fat Body is a Locus of the Sex Difference in Reproductive and Ingestive Response to Energy Deficits** Attilio Ceretti, Jill E Schneider Biological Sciences, Lehigh University

There are sex differences in the reproductive and ingestive responses to energy deficits in many species (reviewed by Schneider et al., 2013), but the loci of these sex differences remain unknown. In previous experiments using Drosophila melanogaster, we found that copulation rate is decreased and time spent with food is increased after food deprivation (FD) in females, but the same traits were significantly less affected by the same level of FD in males. Next we asked whether these sex differences could be prevented by masculinizing particular tissues. We used RNA-interference (RNAi) for the Tra2 gene to masculinize only the nervous system, only the fat body, or all cells in 2X:2A flies. Global masculinization of all cells in the 2X:2A flies masculinized courtship behavior and the reproductive response to FD. Masculinization of only the nervous system fully masculinized courtship behavior, but did not masculinize the ingestive and reproductive responses to FD. The females with masculinized nervous systems significantly decreased courtship rate compared to fed females and males (FD or fed) ($P < 0.01$), and displayed female levels of FD-induced time with food compared to male controls ($P < 0.01$). By contrast, masculinization of the fat body masculinized the reproductive and ingestive response to FD. The 48-h FD females (2X:2A) with masculinized fat bodies showed no significant decrease in copulation rate compared to fed controls, and decreased FD-induced time spent with food compared to female controls and FD males from the same background ($P < 0.01$). Thus, our genetic manipulation of the fat body successfully masculinized the reproductive response to energy deficits of the 2X:2A females. In wild-type females, the availability of oxidizable glucose was both necessary and sufficient for normal copulation rate. We hypothesize that the female-typical response to energy deficit lies outside the nervous systems, perhaps in insulin signaling controlled by the fat body.

281 **Sexual dimorphism in tumor growth requires innate immune cells from the tumor microenvironment and systemic regulation** Xianfeng Wang[1], Hongcun Bao[2], Anindita Barua[2], Yi-Chun Huang[2], Fei Cong[2], Wu-Min Deng[2] [1]Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, [2]Tulane University School of Medicine

Cancer is a systemic disease that impacts the entire body. While interactions between cancerous tissue and other tissues have been observed for a long time, the exact mechanisms by which cancer cells communicate with other tissues to induce systemic effects are still not well understood. Furthermore, many types of cancer exhibit sexual dimorphism in terms of growth. However, the systemic-level mechanisms underlying such dimorphic regulation remain poorly characterized. Using a *Drosophila* tumor model in which Notch signaling is constitutively activated in a transition zone (TZ) of larval salivary gland (SG) imaginal ring (ImR), we found that tumors from female TZ tumors grow up to 2.5 times as large as the male tumors. Notably, wildtype SG ImRs do not exhibit sexually dimorphic growth during development. Our results reveal that the JNK pathway is the key factor causing sexual dimorphic in tumor growth. Leveraging single-cell RNA sequencing, we identified Eiger, a ligand of the JNK pathway originating from hemocytes. Knocking down Eiger in hemocytes led to a reduction in tumor size and sexual dimorphism. Further investigations have demonstrated found Upd2, a cytokine and leptin orthologue, as a downstream target of the JNK pathway, and it is upregulated more significantly in female tumors than male tumors. Tumor size, as well as sexual dimorphism, is reduced when upd2 is decreased in the tumor. Moreover, we demonstrated that the tumor produced Upd2 mimics the adipose tissue derived Upd2, systemically regulates tumor growth through the Dilp2/Insulin pathway. In summary, our findings reveal that the tumor microenvironment, involving hemocytes, underlies sexual dimorphism and the systemic regulation of tumor growth.

282 **Evolutionary and Functional Characterization of Female Control of the Mating Plug in *Drosophila melanogaster*** Jolie

A Carlisle, Rachel M.J. Craig, Nora Brown, Bianca M. Villanueva, Mikaela Matera-Vatnick, Andrew Clark, Mariana F. Wolfner Molecular Biology and Genetics, Cornell University

In many species males transfer seminal fluid proteins (Sfps) and sperm to their mates. Some Sfps are known to mediate essential reproductive processes such as female sperm storage and allow males to exert control over mating outcomes. However, from mammals to invertebrates, females are also known to exert control over their reproduction. In *Drosophila*, females can manipulate the parental contributions of their partners through molecularly controlling the number of sperm stored from a given mating, a likely regulator of this process being the *Drosophila* mating plug (MP). The MP is composed of Sfps, and some female-derived proteins, that coagulate in the *Drosophila* female reproductive tract. Rapid ejection of the MP prevents sperm retention, while delayed ejection of the MP increases the number of sperm that can be stored. Male x Female interactions mediating timing of MP ejection (MPE) are a likely focal point of sexual conflict over control of paternity outcomes.

Using the genetic variation present in the DGRP, we observed that within *D. melanogaster* there are dramatic differences in female MPE timing (>2 fold). We applied a GWAS design to identify both neuronal and oogenesis genes as candidates mediating female MPE. Using flies lacking a germline, we determined that lack of egg production causes delays in MPE, indicative of a connection between female fecundity and sperm storage. Furthermore, using publicly available single-cell atlas of the fly brain and network analysis, we determined that most of our neuronal gene candidates are part of networks that regulate sex-specific behaviors and development . Using RNAi we have validated many of these neuronal candidates' roles in regulating female MPE.

Female-derived MP proteins may also contribute to timing of MPE. We have investigated the evolution of these proteins and discovered that many male and female MP genes are closely paralogous to each other and predicted to interact via rapidly evolving interfaces, suggestive of evolution under sexual conflict. We have traced the evolution of protein families containing these paralogous male and female reproductive proteins and begun to investigate their function. Together our work uses variation between and within *Drosophila* species to investigate the molecular and behavioral mechanisms mediating female control of the mating plug.

283 **Identification of female ovulation-regulating membrane receptors/proteins in *D. melanogaster*** Mengye Yang[1], Melissa White[1], Jennifer Apger-McGlaughon[1], Geoffrey Findlay[2], Ryan Vignogna[1], Christopher Fromme[1], Mariana Wolfner[1] [1]Cornell University, [2]College of the Holy Cross

Female post-mating responses (PMRs), including increased ovulation, are initiated by male seminal fluid proteins (Sfps) and then manipulated by female pathways and molecules. The implementation of the PMR's large-scale homeostatic changes is complicated, requiring the coordination of multiple tissues, including inter-organ signaling between the nervous system, midgut, fat body, and ovary. Ovulation is the process of releasing mature oocytes from the ovary to the uterus, through the oviduct. A male Sfp ovulin stimulates ovulation by activating female octopamine (OA) neuronal signaling in the hours following mating. Despite the key role OA signaling plays in the ovulation regulation, the broader landscape of female molecular mechanisms governing ovulation remains largely unexplored. Here, we aim to identify female membrane receptors and proteins affecting ovulation to elucidate novel signaling pathways involved in ovulation mediation, and significantly advance our understanding of the complex orchestration of ovulation as well as the reproductive dynamics in *Drosophila melanogaster*.

Using male protein ovulin as a probe, we performed evolutionary rate co-variation screens and AlphaFold-Multimer prediction screens to identify ovulation-regulating protein candidates. We narrowed down the candidate list to twelve strong receptors/proteins based on their effect on egg-laying and/or their expression pattern. Subsequent ovulation assays were performed to examine the ovulation rate in female flies subjected to knockdown or depletion of these candidates, shedding light on their potential roles in ovulation and determining if they act through ovulin. For strong candidates displaying neuronal expression, we investigated whether they are expressed in the female reproductive tract neurons using a Gal4 driven by their endogenous promoters, and also carried out pan-neuronal knockdown as well as specific octopaminergic Tdc2 neuronal knockdown of these proteins to ascertain their action location. To date, we have identified five novel female ovulation-regulating proteins with ovulin-dependent or ovulin-independent roles, several of which are required in Tdc2 neurons to influence ovulation.

284 **Structure-expression analysis of different-sized tandem duplicates of the *Alcohol dehydrogenase* locus in *Drosophila melanogaster*** Matt Seltzer, David Loehlin Biology, Williams College

Tandem gene duplicates are important parts of eukaryotic genome structure, yet the phenotypic effects of new tandem duplications are not well-understood. Previous work suggests that tandem duplications containing an entire gene do not always simply double that gene's expression. This study aims to identify factors that influence tandem duplicate expression by comparing multiple duplicates of the same gene produced from a variety of endpoints. Two hypothesized factors of primary interest are 1) cis-regulatory elements, which could influence expression depending on the sequence content of the duplicated

block, and 2) gene proximity, which would depend on size but not sequence content of the duplicated blocks. We apply a new method, Recombinase-Mediated Tandem Duplication (RMTD), to precisely duplicate the *Alcohol dehydrogenase* (*Adh*) gene in *D. melanogaster*, by pairing 5 left-side and 5 right-side breakpoints. We report preliminary analysis of expression (ADH enzyme activity) from flies with ~20 different tandem duplicate blocks. Expression appears to vary primarily with presence/absence of upstream segments in the duplicated block, suggesting a role of undocumented regulatory sites. Accounting for this variation indicates a possible additional role for distance-dependence of gene expression beyond sequence content.

285 **What vs. When: Determining functional differences between canonical histone H3.2 and variant histone H3.3 in post-mitotic cells** Jeanne-Marie E McPherson[1], Robert E Duronio[2], Daniel McKay[2] [1]Genetics, University of North Carolina at Chapel Hill, [2]Genetics, Biology, University of North Carolina at Chapel Hill

Histone proteins organize DNA into chromatin, which regulates all DNA-dependent processes. The genome encodes two types of histones: canonical histones that are expressed only during S-phase, and variant histones that are expressed throughout the cell cycle. Canonical histone H3.2 and variant histone H3.3 are some of the most highly conserved proteins among eukaryotes, suggesting that each histone type performs unique functions. Despite this conservation, it is not known if variant H3.3 function is mediated by its unique protein sequence or by its cell-cycle independent expression. Canonical H3.2 and variant H3.3 differ by just four amino acids. Three of these residues mediate interactions with histone chaperones and thus influence where H3 types are deposited in the genome. The fourth residue at position 31 can be post-translationally modified on H3.3, and this phosphorylation has been linked to gene regulation. To uncouple H3 protein identity from timing of expression, we generated *D. melanogaster* CRISPR mutants that express H3.2 from the endogenous *H3.3* genes ($H3.3^{H3.2}$). Because the adult brain is primarily composed of post-mitotic neurons that accumulate replication-independent histones, we examined the effects of $H3.3^{H3.2}$ mutations on adult behavior and genome organization in brains. We found that $H3.3^{H3.2}$ mutants have reduced lifespan and behavioral defects. Notably, these defects are rescued by expressing one copy of wild-type *H3.3,* indicating that the H3.3 protein is required for normal post-mitotic cell function. Mutation of the H3.3-specific residue S31 had no impact on lifespan and mild behavioral defects, demonstrating that loss of S31 is not the main driver of $H3.3^{H3.2}$ mutant phenotypes. An important corollary to this finding is that interactions between each histone type and their respective chaperones are critical for normal genome function. To understand how these interactions impact genome organization, we performed ATAC-seq in 1-day and 10-day old $H3.3^{H3.2}$ and $H3.3^{S31A}$ mutant brains. We found that although overall patterns of chromatin accessibility do not substantially change in either mutant at either age, quantitative differences in accessibility are detectable at many locations. We are testing whether these changes in chromatin accessibility affect gene expression. Our work is revealing how histone type contributes to control of genome function.

286 **Impact of *de novo* transposition events on the 3D nuclear architecture and sequence structure of telomeres and centromeres in multigenerational heat-shock lines of *Drosophila melanogaster*** Ryan Pellow[1], John Atagozli[1], Josep Comeron[1,2] [1]Department of Biology, University of Iowa, [2]Interdisciplinary Program in Genetics, University of Iowa

Transposable elements (TEs) make-up a substantial portion of most eukaryotic genomes. These “jumping genes” or transposons are key drivers of evolutionary innovations and adaptations, often as a result of their functional motifs altering gene regulatory programs. Advances in chromosome conformation capture methodologies (e.g., Hi-C) have also allowed detecting significant associations between TE presence and the looping mechanisms of chromatin which in turn shape the 3D nuclear organization. The ability to capture the impact and dynamics of TEs on genomic properties, however, is limited by the difficulty to capture *de novo* insertions and characterize the before-and-after in the same genetic background. To address this challenge, we have generated long-term heat-shock mutation accumulation lines (HSMAL) from different isogenic strains of *Drosophila melanogaster*, where virgin 2-day old females were exposed to moderate heat stress (36.5°C for 20 minutes) before mating. Over the course of 50 generations of heat-shock, we have performed DNA-seq (both Illumina short-read and Nanopore ultra-long read), total RNA-seq, and Hi-C. By leveraging the DNA-seq data, we have been able to generate HSMAL specific references, map *de novo* TEs and quantify transpositions bursts genome-wide. The RNA-seq data confirms transposition bursts resulting from increased transcriptional activity of specific TEs, while also highlighting the direct and indirect impacts of TE insertions on the transcriptome. We also demonstrate the ability of *de novo* TEs to both create and disrupt topologically associated domain (TAD) boundaries using Hi-C data. Finally, our NanoPore sequencing data identifies not only centromeric and telomeric sequence variation between natural strains of *D. melanogaster*, but also changes resulting from the generational heat-shock.

287 **Investigating the role of the microbiome on gonadogenesis** Taylar J Mouton, Meg Susil, Nichole Broderick Biology, Johns Hopkins University

Animal gut microbes have been shown to impact most aspects of host physiology. In humans, these microbes, or microbiome, contribute to a growing number of host disease states. However, the high complexity of the human microbiome makes it

harder to isolate variables and define mechanisms. Additionally, it is nearly impossible to study how the previous generation's microbiome status impacts their offspring and subsequent generations. Thus, model organisms, such as Drosophila melanogaster have provided a valuable tool to study host-microbiome interactions. The D. melanogaster microbiome has relatively low-complexity and the microbes can be readily cultured. Interestingly, some studies have reported that parental microbiome status can exert unexpected consequences on offspring development and physiology, spanning for multiple generations. While these studies indicate the microbiome impacts host physiology and development intergenerationally, they have attributed different bacterial strains and signaling pathways to these impacts. Therefore, it is still unknown how exactly the microbiome could be impacting early development to contribute to the faithful maintenance of processes like oogenesis and early embryogenesis. The goal of this research was to characterize the impact of the microbiome on D. melanogaster fecundity. To this aim, we've measured the fecundity of axenic or microbiome-free flies compared to conventionally reared flies that have their microbiome. We found that while the total number of eggs laid by axenic and conventionally reared female flies over a 30-day period does not significantly differ, the daily trajectory of eggs laid by conventionally reared flies to axenic flies is significantly different, with axenic flies laying more eggs than their conventionally reared counterparts as they age. Additionally, immunofluorescence data indicates that at the cellular level there are differences between gonads of axenic and conventionally reared flies. These results together demonstrate the importance of assaying a female's egg laying lifespan when assaying their fecundity, especially when comparing conventionally reared and axenically reared flies.

288 **Quantifying dynamics of optogenetic signaling activators in zebrafish embryos** Allison Saul[1], Patrick Müller[2], Katherine Rogers[3] [1]NICHD, National Institutes of Health, [2]Universtität Konstanz, [3]National Institutes of Health

Embryonic cells are thought to decode signaling intensity, duration, and dynamics to choreograph cell fate decisions. To investigate how different signaling features drive fate, we are developing optogenetic tools that use light to independently manipulate these features in transparent zebrafish embryos. Effective application of these tools requires an understanding of how optogenetically controlled signaling pathways respond to light exposure: how strongly, how quickly, and for how long? To address these questions, we are reading out optogenetically activated BMP, Nodal, and FGF signaling using western blotting or immunofluorescence staining. To avoid problematic, inadvertent light exposure during sample processing, we fix embryos to effectively "freeze" them in time prior to carrying out either assay. For the western protocol, we have adapted a method to liberate protein from fixed samples using Tris, SDS, and heat. We plan to collect samples before, during, and after light exposure to quantify the on/off kinetics of our optogenetic tools. In addition, we will expose embryos to different light intensities to determine the relationship between light intensity and signaling levels. This characterization will enable us to properly apply our tools to provide insight into how signals are interpreted by cells to choreograph differentiation and fate decisions. Funding: NIH Intramural ZIAHD009002-01 to KWR.

289 **Single-cell analysis of shared signatures and transcriptional diversity during zebrafish development** Abhinav Sur[1], Yiqun Wang[2], Paulina Capar[1], Gennady Margolin[3], Morgan K Prochaska[1], Jeffrey A Farrell[1] [1]Division of Developmental Biology, National Institute of Child Health and Human Development, National Institutes of Health, [2]Department of Molecular and Cellular Biology, Harvard University, [3]Bioinformatics and Scientific Programming Core, National Institute of Child Health and Human Development, National Institutes of Health

During development, animals generate distinct cell populations with specific identities, functions, and morphologies. We mapped transcriptionally distinct populations across 489,686 cells from 62 stages during wild-type zebrafish embryogenesis and early larval development (3–120 hours post-fertilization). Using these data, we identified the limited catalog of gene expression programs reused across multiple tissues and their cell-type-specific adaptations. We also determined the duration each transcriptional state is present during development and identify unexpected long-term cycling populations. Focused clustering and transcriptional trajectory analyses of non-skeletal muscle and endoderm identified transcriptional profiles and candidate transcriptional regulators of understudied cell types and subpopulations, including the pneumatic duct, individual intestinal smooth muscle layers, spatially distinct pericyte subpopulations, and recently discovered *best4*+ cells. To enable additional discoveries, we make this comprehensive transcriptional atlas of early zebrafish development available through our website, Daniocell. Funding: NIH Intramural ZIAHD008997 to JAF.

290 **Safe harbor landing sites for reproducible transgenesis and variant testing in zebrafish** Robert L. Lalonde[1], Harrison H. Wells[1], Cassie L. Kemmler[1], Susan Nieuwenhuize[1], Raymundo Lerma[1], Alexa Burger[1], Christian Mosimann[2] [1]Department of Pediatrics, Section of Developmental Biology, University of Colorado School of Medicine, Anschutz Medical Campus, [2]University of Colorado School of Medicine, Anschutz Medical Campus

Transgenic zebrafish strains are a major tool for biomedical research, enabling live imaging of developmental processes, cell lineage tracking, mechanistic studies, and gene variant testing in a vertebrate model *in vivo*. However, routine transgenesis in zebrafish with random DNA integration via Tol2 or I-SceI presents considerable challenges: the creation of high-quality, single-

copy transgene integrations with reproducible activity remains time-, labor-, and resource-intensive and faces unpredictable variability due to chromatin effects. Targeted vector integration into validated genomic loci using phiC31 integrase-based *attP*/*attB* sequence recombination has transformed mouse and *Drosophila* transgenesis. While phiC31 functions in zebrafish, validated genomic loci carrying *attP*-based landing or safe harbor sites validated for a wide variety of transgene constructs remain missing.

Here, using CRISPR-Cas9, we converted two well-validated Tol2-based zebrafish transgenes (*ubi:Switch*, *hsp70l:Switch*) to *attP* landing site alleles we call *phiC31-Integrase Genomic Loci Engineered for Transgenesis* (*pIGLET*). Generating reporters and effector transgenes in landing sites *pIGLETa* and *pIGLETb*, we document their suitability for broad transgenesis applications. Re-creating transgenic reporters that are highly susceptible to position effects including Cre-responsive *loxP* switches, we demonstrate that both landing sites are well-suited to predictably generate transgenes with faithful activities in different developmental cell lineages, organs, and Cre/*lox* applications. For both landing sites, we routinely achieve germline transmission of targeted transgene integrations with 50-90% efficiency, drastically reducing resources and animal numbers needed to generate individual transgenic lines. We document that phiC31-based transgenesis into *pIGLETa* and *pIGLETb* results in representative reporter expression patterns already in injected F0 embryos, enabling injection-based screening for reporter activity and germline validation such as for gene-regulatory element discovery. We show that disease-associated enhancers can be directly compared to reference enhancer activity, enabling qualitative and quantitative enhancer testing.

Altogether, our *pIGLET* landing sites with associated protocols and plasmids enable community-accessible, reproducible, and targeted transgenesis for numerous applications while greatly reducing the workload of generating new transgenic zebrafish lines.

291 **ABE-Ultramax for high-efficiency biallelic adenine base editing** Wei Qin[1], Fang Liang[2], shengjia Lin[3], Cassidy Petree[3], Kevin huang[3], yu zhang[2,2], lin li[2], Pratishtha Varshney[3], Philippe Mourrain[4], yanmei liu[2], Gaurav Varshney[3] [1]gene and human disease, Oklahoma medical research foundation, [2]South China Normal University, [3]Oklahoma Medical Research Foundation, [4]Stanford University

Advancements in repurposing CRISPR-Cas9 technology, especially the development of base editors, have fundamentally reshaped the field of genetic variant research. When paired with tractable model organisms, such as zebrafish, these base editors accelerate and improve the accuracy of *in vivo* functional analyses of genetic variations. However, base editors are restricted by protospacer adjacent motif (PAM) sequences and specific editing windows, hindering their applicability to a broad spectrum of genetic variants. They can also induce unintentional bystander mutations and tend to have lower editing efficiencies in living organisms compared to cultured cell lines. To overcome these challenges, here we focused on adenine base editors (ABEs), which have the potential to correct approximately half of all pathogenic human point mutations, and engineered a suite of ABE variants that we call ABE-Umax with high editing efficiency and a low frequency of insertions and deletions (indels) in zebrafish. Within the ABE-Umax platform, we present ABEs with shifted, narrowed, or broadened editing windows, reduced bystander mutation frequency, as well as highly flexible PAM sequence requirements. These innovative base editors promise to overcome many limitations of their predecessors, revolutionizing disease model generation and the broader field of gene therapy.

292 **Discovering context-specific functionally-equivalent genes in research organisms using cross-species transcriptome-based machine learning** Hao Yuan[1], Christopher A Mancuso[2], Kayla Johnson[3], Ingo Braasch[4], Arjun Krishnan[3] [1]Genetics and Genome Science, Michigan State University, [2]Department of Biostatistics & Informatics, University of Colorado Anschutz Medical Campus, [3]Department of Biomedical Informatics, University of Colorado Anschutz Medical Campus, [4]Department of Integrative Biology, Michigan State University

Research organisms are quintessential for performing functional experiments, disease modeling, and drug testing of human disorders. However, evolutionary divergence between humans and research organisms complicates effective knowledge transfer across species, hindering the identification of appropriate gene targets in research organisms for studying human diseases. Common strategies use homology as a bridge for knowledge transfer, but homology does not guarantee equivalent function across species. Moreover, as the roles of genes is likely to vary across disease contexts, functional equivalence between genes across species is likely to be highly context-specific. While a number of computational methods have been developed to transfer knowledge across species beyond solely using gene homology, they do not account for disease context in knowledge transfer. To address this challenge, we have developed a novel approach to infer disease-specific genome-scale gene networks simultaneously in humans and research organisms using hundreds of thousands of existing transcriptomes in multiple species. Disease-specific signals in these massive public data are captured by a regression-based approach that identifies minimal sets of transcriptomes in human and research organisms that can recapitulate disease transcriptome(s) from patients. Then, we compare the resulting networks in human and research organisms to predict genes that are likely to

be functionally-equivalent across species based on the high similarity of their local network neighborhoods. We demonstrated the power of our approach by applying it to discover functionally-equivalent genes in zebrafish. However, our approach can be applied to any research organism with sufficient existing public transcriptomics data. Our method helps to simplify the process of selecting a well-suited organism and gene targets for functional tests. Additionally, functionally-equivalent genes identified by our approach could be integrated to Alliance of Genome Resources, offering invaluable guidance for functional tests and benefiting disease model communities. To showcase the power of our method, we conducted a case study on Facioscapulohumeral Muscular Dystrophy (FSHD) - a rare genetic muscle disease caused by heterogeneous genetic factors. We investigated potential pathogenic genes related to FSHD and found functional equivalents in research organisms.

293 **Towards large-scale computational whole-organism microanatomical phenotyping: Democratizing access to large-field submicron histotomography, cloud-based visualization, and computational workflows** Keith C Cheng[1], Daniel Vanselow[2], Michael Morehead[3], Alex Y Lin[2], Patrick J La Riviere[4], Sharon Xiolei Huang[5], Khai Chung Ang[2], Mahmut T Kandemir[6] [1]Pathology, Penn State College of Medcine, [2]Pathology, Penn State College of Medicine, [3]Isovisio, [4]Radiology, University of Chicago, [5]Information Sciences and Technology, Pennsylvania State University, [6]Computer Science, Pennsylvania State University

Histopathology is a traditionally 2-dimensional form of tissue diagnosis based on histology, whose essential features include undistorted (isotropic) and single micron resolution (equivalent to submicron digital resolution), unbiased (pan-cellular) representation of all cell types, and the ability to image sample slices more than a centimeter in largest dimension. Reverse translation of that power to the 3D realm would enable volumetric and cell population studies that addresses the model system community goal (https://doi.org/10.1242/dmm.049600) of whole-organism phenotyping for genetic phenome projects focused on mutants and chemical phenome projects focusing on treated organisms. Systems consideration of alternative 3D imaging technologies led us to focus on the development of quantitative, 3D, whole-organism phenotyping, based on the development of a 3D form of histology representing a customized form of micron-scale CT, call histotomography.

To democratize accessibility, we are working with the DOE and the University of Chicago to implement synchrotron-based histotomography for the community. To address user visualization of TB-range 3D image files, we have implemented cloud-based multi-planar visualizations on common commuters using Neuroglancer. Streaming from servers to allow cloud-based Virtual Reality visualization on normal computers is also possible.

Reaching the aspirational goal of complete, quantitative, microanatomical phenotyping in whole centimeter-wide genetic model organisms and their tissues will require community support for democratized access to histotomography. Analogous to posting of DNA sequence for the creation of bioinformatic tools, we are also working towards incorporating the goals of computational phenotyping into a Galaxy-based system of access to collections of reference and experimental images, the above cloud-based visualization capabilities and computational workflow systems designed to foster community-based computational tool development. Finally, the pan-cellular nature of whole-organism microCT/histotomography suggests their potential use as cloud-based anchors for histology and other multimodal and multi-scale images, as well as large-scale molecular analytics (spatial biology).

294 **Telomere-to-telomere genome assemblies for commonly used zebrafish laboratory strains** Javan O Okendo[1], Sergey Koren[2], Arang Rhie[2], Aranza Torrado[1], Joy M Murphy[3], Zoltan Varga[3], Adam M Phillippy[2], Shawn M Burgess[1] [1]Translational and Functional Genomics branch, National Human Genome Research Institute, [2]Computational and Statistical Genomics Branch, National Human Genome Research Institute, [3]Institute of Neuroscience, 1254 University of Oregon Eugene

The most recent zebrafish reference genome assembly was released in May of 2017. At the time, it was generated with the state-of-the-art sequencing technologies in combination with a high-density genetic map and BAC libraries made from the Tübingen line (TU). The assembly consists of 19,725 contigs assembled into 1,917 scaffolds. Recent advances in both “long-read” sequencing technologies and genome assembly algorithms have made “complete” genome assemblies possible for the first time as has been shown by the recent release of a fully assembled human genome. We have devised two separate strategies for assembling the zebrafish genome “telomere-to-telomere” (T2T): The first strategy uses homozygous TU fish generated by heat shock disruption of mitosis **I**. Cell lines were established for two HS diploid fish and then genomic DNA was isolated both from the adult tissues as well as the cultured fibroblasts. DNA from the tissue was used for PacBio HiFi sequencing and the fibroblast DNA was used for Oxford Nanopore sequencing (ONT). We performed *de novo* assembly using the Verkko genome assembler. The second strategy we termed the “3 generations” approach. One fish from each of the 4 most commonly used zebrafish lab lines: TU, AB, WIK, and TL were used as the 4 “grandparents” and were short read sequenced to document all unique, identifying SNV for each parent. One fish from each of the 2 grandparent pairings was used to generate pools of the 3rd generation offspring. The pooled genomic DNA from these offspring was used for both PacBio HiFi and ONT sequence. The SNV data from the grandparents is then used to separate all the reads into haplotype bins and all 4

haplotypes are resolved simultaneously using the Verkko assembler. The data for strategy I has a gapless assembly of 1.45GB in 25 chromosomes and an additional ~48 million bases of new sequence has been added to zebrafish reference genome. The strategy 1 genome assembly is more contiguous with N50 of 58 MBs and L50 of 11. Our strategy II shows that TU, AB, WIK, and TL zebrafish strains have varied number of unique k-mers and they exhibit different levels of heterozygosity. We hope this new assembly will open new frontiers in zebrafish genetic research.

295 **The genetic basis of copper resistance and adaptation in natural populations** Elizabeth Everman[1], Stuart Macdonald[2], John Kelly[2] [1]University of Oklahoma, [2]University of Kansas

Heavy metal pollution has pervasive environmental, health, and evolutionary impacts. In humans, health risks range from permanent neurological disease to increased morbidity of degenerative syndromes. Our previous work has demonstrated that variation in susceptibility to metal toxicity is influenced by a combination of genetic variation, environmental exposure, and their interaction. Investigation of the evolutionary processes that lead to complex trait variation has great biomedical significance as we seek to understand the gene-by-environment interactions, genetic constraints, and genetic risk factors that contribute to increased susceptibility to toxic heavy metal exposure in human populations. We focus on copper given the important role this metal plays as an environmental contaminant and as a critical micronutrient for normal physiological development and maintenance in most organisms. Recently, we used *Drosophila melanogaster* to examine natural variation in metal resistance by collecting wild flies from four populations with varying exposure risk to metal pollution, focusing on the potential evolutionary impact of mining and agricultural activities. Copper resistance varied among the wild populations, increasing in sites that have been previously subject to intense mining and agricultural activity. Using whole genome sequencing and bulked segregant analysis, we identified 288 SNPs distributed across the genome that contribute to copper resistance. Most SNPs had population-specific effects, suggesting that the effects of alleles associated with increased resistance are influenced by unique evolutionary histories as well as population-specific genetic backgrounds. SNPs with consistent effects on copper resistance were also detected, calling attention to candidates potentially involved in parallel adaptation. SNPs mapped to several novel candidates as well as to the known copper homeostasis gene *CG11825*, providing new insight on the collection of genes that influence resistance in natural populations. This work demonstrates the highly polygenic nature of copper resistance in natural populations is subject to complex interactions between genetic background and evolutionary history. Current work seeks to investigate these evolutionary patterns further through a long-term artificial selection experiment. Using an integrative approach that leverages whole genome and RNA sequencing and experimental evolution, we will track the dynamic shifts in allele frequency and gene expression in response to copper stress in diverse naturally derived genetic backgrounds. By focusing on multiple physiological and behavioral traits, these approaches will provide critical insight into the interconnectedness of multiple response traits, while also illuminating genetic factors that influence behavioral and learning disabilities linked to metal poisoning.

296 **Characterizing Epistasis in Copper Sulfate Resistance using Chromosome Fixation and Bulk Segregant Analysis** Cassandra Buzby, Federica FO Sartori, Mark Siegal New York University

Complex traits are the products of multiple genetic and environmental factors, yet how these influences interact largely remains a mystery. The contribution of genetic interactions to natural trait variation is particularly challenging to estimate experimentally, and current approaches for detecting epistasis are often underpowered. Powerful mapping approaches such as bulk segregant analysis, wherein individuals with extreme phenotypes are pooled for genotyping, obscure epistasis by averaging over genotype combinations. To accurately characterize and quantify epistasis within natural trait variation, we have engineered Saccharomyces cerevisiae strains to enable crosses in which one parent's chromosome is fixed while the rest of the chromosomes segregate. We then use bulk segregant analysis to identify quantitative trait loci whose effects depend on alleles on the fixed parental chromosome, indicating a genetic interaction with that chromosome. Using this method, we can identify interacting loci with high statistical power.

We applied this approach to a cross of a yeast strain derived from a wine barrel ("Wine") and a strain derived from an oak tree ("Oak"), from which we obtained large pools of segregating progeny fixed for chromosomes I or VIII from either parent. We tested resistance to copper sulfate, as copper is a common stressor in wild yeast; these strains show a marked difference in survival in the presence of copper, allowing us to compare variation in natural populations in a trait relevant in their different environments. We identify effects dependent on the genetic background as well as changes in additive effects resulting from removal of variation in the CUP1 locus, which encodes a metallothionein and has a large association with copper resistance. We demonstrate that this technique may be used to tease apart the roles of additive quantitative trait loci in the broader context of the genome as well as detecting loci which interact with each fixed chromosome. Our results point to further complexity of genetic architecture than previously appreciated in the trait of copper resistance, revealing specific interactions between multiple genetic factors.

297 **A molecular evolutionary cascade facilitates nematode parasitism of prey carrying toxic cardiac glycosides** Perla Achi[1], Adler Dillman[1], Simon Groen[1], Anil Baniya[1], Peter Douglas[2], Connor Goldy[1], Damaris Godinez-Vidal[1] [1]Nematology, UCR, [2]Molecular Biology, UT Southwestern Medical Center

Target-site insensitivity (TSI) is an important mechanism of animal resistance to natural and man-made toxins. TSI evolved in parallel in the monarch butterfly and other insects that specialize on milkweeds and is thought to have facilitated sequestration of cardiac glycosides (CGs) that may protect these insects from predation and parasitism. Substitution N122H in the CG-binding pocket of the molecular target, the Na+/K+-ATPase alpha subunit (ATPα), strongly enhances TSI and evolved in parallel in CG-sequestering insects across six orders. Upon performing a genetic screen of the Na+/K+-ATPase, we recently identified N122H in the entomopathogenic nematode (EPN) *Steinernema carpocapsae*, which parasitizes insects around milkweeds. This sets up the possibility that parallel evolution of N122H may not only have facilitated CG sequestration by insects, but also nematode parasitism of CG-carrying insects. Here, we show that N122H is rare among nematodes and that, among species tested for CG tolerance, *S. carpocapsae* showed significantly stronger insensitivity to diverse CGs than nematodes without N122H, including free-living *Caenorhabditis elegans* and parasites of milkweed roots. CRISPR gene editing in *C. elegans* showed N122H is sufficient for overcoming toxicity of CG levels found in sequestering insects. However, N122H was accompanied by costs related to nervous system robustness, potentially explaining its rarity among nematodes. Finally, *S. carpocapsae* was the only EPN tested that was highly successful at infecting CG-carrying insects and that displayed attraction to CGs. Taken together, our results suggest that a molecular evolutionary cascade of parallel substitutions across hosts and parasites, last sharing common ancestry 600 million years ago, may shape multitrophic interactions.

298 **Chaos below the stability: evolution-selection equilibrium model explains co-existence of resistant and susceptible strains** Pleuni S. Pennings Biology, San Francisco State University

Drug resistance is a problem in many pathogens, including viruses, bacteria, fungi and parasites. While overall, levels of resistance have risen in recent decades, there are many examples where after an initial rise, levels of resistance stabilize. The stable co-existence of resistance and susceptibility has proven hard to explain – in most models either resistance or susceptibility ultimately “wins” and takes over the population. Here we propose a simple stochastic model, akin to mutation-selection balance theory, which can explain several key observations about drug resistance: (1) the stable coexistence of resistant and susceptible strains (2) at levels that depend on drug usage (3) and the observation that resistance is often carried by many different resistant strains. It predicts that many resistant strains should continuously appear (due to positive selection within treated hosts) and disappear (due to competition with susceptible strains in the rest of the population). When resistance is due to horizontally transferred genetic elements, the model predicts that strains continuously acquire these genetic elements. We used data on 40,000 *E. coli* strains to test this prediction for a resistance mutation and two resistance genes in the UK and found that the data are consistent with the predictions.

299 **Investigating freeze-thaw tolerance in *S. cerevisiae* via experimental evolution** Leah Anderson[1], Liya Miksovsky[2], Maitreya Dunham[1] [1]Genome Sciences, University of Washington, [2]Yale University

Although freezing and thawing is commonplace for *S. cerevisiae* both in the laboratory and natural environments, cellular mechanisms for freeze-thaw tolerance remain understudied. The main goal of my project is to elucidate genetic factors that play a role in freeze-thaw tolerance and functionally characterize variation in those loci.

I conducted experimental evolution for yeast freeze-thaw tolerance by subjecting initially clonal yeast populations to strong selective pressure via exposure to serial rounds of freezing and thawing. After 20 cycles of freeze-thaw evolution, increased freeze-thaw tolerance was observed in 34 independent populations. The evolved populations were then whole-genome sequenced and analyzed for SNPs, copy number variation, and transposons that differ from the ancestral genome. We found multiple independent mutations in cell wall biosynthesis proteins, components of the RAM signaling pathway, and factors required for proper cell budding. My next steps are to verify the precise effects these mutations have on phenotype by isolating individual mutations and measuring their effects on freeze tolerance as well as other phenotypes, including cell wall integrity, budding capabilities, osmotolerance, and heat sensitivity. Preliminary data has shown that tradeoffs previously thought to exist between freeze-thaw tolerance and osmotolerance are not ubiquitous across freeze-thaw evolved yeast clones. This underscores the value of experimental evolution as a method for identifying freeze-thaw tolerance mechanisms, as it uncovers multiple genetic pathways for tolerance, some which may not exhibit previously associated tradeoffs.

This work will provide a catalog of freeze-thaw tolerance genes and provide better understanding of how changes in the genome impact survival to extreme temperature changes. Furthermore, freeze-thaw tolerant yeast generated via experimental evolution have potential for utility in both biomedical research and industrial applications, as well as providing a better understanding of how organisms in the natural world adapt to frequent temperature fluctuations in a changing global

environment.

300 **Natural hybrid zones reveal the molecular underpinnings and evolutionary history of hummingbird pollination in *Penstemon*** Lucas C Wheeler, Joshua TE Stevens, Noah H Williams, Carolyn Wessinger Biological Sciences, University of South Carolina

The hummingbird pollination system has evolved multiple times in the genus *Penstemon* from a bee-pollinated ancestral state. In section Habroanthus, despite clear signals of hybridization between species, we have found strong evidence of four convergent acquisitions of the so-called hummingbird pollination syndrome. This phenotype is characterized by long-tubed red flowers with abundant dilute nectar and exserted stamens and styles, and is predictive of hummingbird visitation in *Penstemon*. By performing genetic crosses with a bee vs. hummingbird pollinated sister species pair in section Habroanthus, we have identified regions of the genome that are tightly associated with the characteristic components of the hummingbird syndrome. QTLs include genes associated with the control of floral pigmentation and morphology. But, have these same genetic mechanisms been replicated during the evolution of the hummingbird-pollinated phenotype in other Habroanthus lineages? To answer this question we are taking advantage of a collection of hybrid zones that occur between another hummingbird vs. bee pollinated sister pair in Habroanthus: *P. eatonii* and *P. laevis*. We have performed phenotyping and whole-genome resequencing of a large sample of hybrid and parent individuals from three separate populations each. This dataset has allowed us to make use of admixture mapping to identify loci that are associated with the relevant components of the hummingbird pollination phenotype, and provides a detailed window into variations in local ancestry and the history of introgression in the hybrid zones. We find evidence that parallel evolutionary changes in at least one locus, the anthocyanin pathway branching enzyme *Flavonoid* 3',5'-*hydroxylase* (F3'5'H), occurred in both of the studied sister pairs. However, our results suggest that the molecular mechanisms underlying the other components of the hummingbird-pollinated phenotype differ along parallel lineages.

301 **Cracking coevolution: consequences of spatial dynamics on coevolving quantitative traits** Victoria Caudill, Peter Ralph University of Oregon

Coevolution between two species can lead to exaggerated phenotypes that vary in a correlated manner across space. However, the conditions under which we expect such spatially varying coevolutionary patterns in polygenic traits are not well-understood. I investigate the evolutionary dynamics of coevolution of two species undergoing reciprocal adaptation across space and time, using both theory and simulations inspired by the newt-snake system. In this system, the amount of tetrodotoxin produced by Taricha granulosa (rough-skinned newt) is strikingly correlated with resistance of one of their predators, Thamnophis sirtalis (garter snake), to this toxin. I explore how the genetic architecture of the toxin and resistance traits affects the coevolutionary dynamics by manipulating the mutation rate and the effect size of mutations, demonstrating how different genetic architectures lead to different coevolutionary outcomes in geographically explicit settings with both homogeneous and nonhomogeneous environments. I find that intermediate trait polygenicity often provides an advantage in the coevolutionary race, suggesting that there might be an optimal combination of mutation rate and effect size in the coevolutionary arms race. Furthermore, I only found strong spatial correlations between traits with heterogeneous environments, suggesting that intrinsic coevolutionary fluctuations are not sufficient to explain observed correlations.

302 **The expanded role of the conserved *snpc-1* and *snpc-3* gene families in *C. elegans* small RNA transcription** Lars Benner, Rebecca Tay, Margaret Starostik, Ayaka Inoki, Mindy Clark, Michael Schatz, John Kim Biology, Johns Hopkins University

PIWI-interacting RNAs (piRNAs) are a class of small RNAs that have a conserved function in protecting the germline genome from the deleterious effects of mobile DNA elements. In repressing these elements, piRNAs preserve the integrity of the genome and ensure its faithful transmission to the next generation. While the transposon silencing function of piRNAs is well understood, the transcriptional regulation and sexual dimorphic expression of piRNAs remain largely unknown. The conserved snRNA activating protein complex (SNAPc) is a well-established transcription factor complex that drives small nuclear RNA (snRNA) transcription. In flies and mice, the SNAPc holocomplex consists of SNPC-1, SNPC-3, and SNPC-4 subunits, which are each encoded by a single gene. In contrast, the *C. elegans snpc-1* and *snpc-3* genes have been amplified through gene duplications to comprise several paralogs, each with distinct roles. We previously showed that the SNPC-1 family protein SNPC-1.3 is a male piRNA transcription factor expressed in the male germline. Here, we provide biochemical and genetic evidence to show that the SNPC-1 paralog SNPC-1.2 constitutes a novel female piRNA transcription factor. Additionally, genetic knockout and RNAi-based knockdown assays reveal that the *snpc-3* family genes *snpc-3.1* and *snpc-3.2* comprise functionally redundant core piRNA transcription factors required for the transcription of both male and female piRNAs, while *snpc-3.4* is uniquely involved in snRNA transcription. Collectively, the *snpc-1* and *snpc-3* gene families encode specificity factors for three distinct protein complexes that discriminate between the transcription of sex-specific piRNAs and snRNAs in the *C. elegans* genome. Our work provides insights into the sexually dimorphic, piRNA-mediated regulation of germline genes to maintain proper germline development and suggests that piRNA biogenesis emerged from the diversification of the ancient

snRNA transcriptional machinery.

303 **Determination of the mechanisms governing piRNA degradation** Benjamin Pastore, Hannah L Hertz, Wen Tang Biological Chemistry and Pharmacology, The Ohio State University

Organisms are constantly under attack by foreign nucleic acids from viruses and transposons that cause genome damage and an array of genetic diseases. To combat these foreign genetic elements organisms evolved RNA- and DNA- directed immune pathways such as RNA-interference (RNAi) and CRISPR/CAS. One of the most ancient and evolutionarily conserved RNA-directed immune pathways is the PIWI/piRNA pathway. In animal germlines piRNAs, together with effector PIWI proteins, suppress transposons and are indispensable for fertility. Since their discovery, piRNA biogenesis and function have been well studied, yet until recently the mechanisms governing piRNA degradation were largely unexplored. In a recent work, we identified a piRNA quality control mechanism that is mediated by 3' nontemplated nucleotide additions (3' tailing) of RNA nucleotides. Moreso, we found that piRNA::target interactions have the capacity to induce 3' uridine (U) tailing of piRNAs and their degradation. Using genetics, high-throughput RNA sequencing, and bioinformatics, we have identified and characterized the enzymes required for piRNA 3' U tailing and degradation. Our findings suggest that piRNA 3' U tailing is concurrent, but not required for piRNA degradation. Additionally, our investigation elucidated the mechanism by which different pools of sex-specific piRNAs are regulated throughout development in *C. elegans*. In all, this work expands our knowledge of both how and why piRNAs are degraded by identifying and characterizing the proteins involved in piRNA 3' tailing and degradation, as well as assigning a physiological function of piRNA decay.

304 **Polarizing Epithelia with Branched Actin Regulators** Patricia Irizarry[1], Luigy Cordova-Burgos[2], Martha Soto[2] [1]Pathology, Rutgers Univeristy, [2]Pathology, Rutgers University

Protein trafficking helps maintain epithelial polarity, and it likely plays a role establishing epithelial polarity during development. Branched actin networks support epithelial polarity, in part by maintaining polarized protein transport. For example, loss of the branched actin regulating WAVE complex results in apical/basal defects of epithelia, including mislocalization of the Cadherin protein, a major regulator of apical/basal polarity (Cordova-Burgos et al., 2020). WAVE and Cadherin colocalize at key transport organelles, including RAB-11-positive recycling endosomes, and at the Golgi. Loss of WAVE leads to slower and mis-directed movements of the apically targeted RAB-11-positive endosomes (Cordova-Burgos et al., 2023). WAVE and Cadherin also colocalize and work together to polarize events during embryonic development (Sasidharan et al., 2018). Therefore, understanding how branched actin contributes to polarized events is essential for understanding epithelial polarity. However, in *C. elegans*, only two Nucleation Promoting Factors (NPSs) that regulate branched actin through Arp2/3 have been examined, WAVE and WASP. We therefore analyzed the orthologs of the third *C. elegans* Apr2/3 NPF, the WASH complex. The WASH complex is similar to the WAVE complex, in that both complexes include 5 paralogous components. Surprisingly, only 4 or the 5 components had been identified in *C. elegans*, and the role of WASH in trafficking had not been described. We used existing mutations, RNAi and CRISPR to determine the effects of loss of the WASH complex, focusing on two epithelial tissues: the adult intestine and the embryonic epidermis. We used our phenotypic assays to test a candidate for the missing 5th WASH component, the FAM21 proposed ortholog, CO5G5.2. Loss of any WASH component, using mutations or RNAi, resulted in the same phenotype: defective transport of cargo on RAB-5 and PI(3)P-positive early endosomes. This result suggests *C. elegans* WASH regulates sorting during endocytic recycling, through retrograde trafficking to the Golgi apparatus. Loss of WASH components also resulted in embryos that die during morphogenesis. Therefore, *C. elegans* has a conserved, 5-member WASH complex that appears to function, like its orthologs, in early endosome to Golgi retrograde transport. Our in vivo analysis of WASH function in two different times and places during development will allow us to determine conserved and divergent features of the *C. elegans* WASH complex.

305 **Investigating the Impact of PI(4,5)P_2 on Biogenesis of Extracellular Vesicle Subpopulations from *C. elegans* Cilia** Malek Elsayyid, Alexis Semmel, Jessica E Tanis Biological Sciences, University of Delaware

Extracellular vesicles (EVs) are membrane-enclosed structures that transfer bioactive proteins, RNAs, and metabolites to recipient cells. Secreted from nearly all cell types, EVs play critical roles in physiological processes and pathological conditions. In *C. elegans*, EVs are shed from primary cilia, which are specialized microtubule-based organelles that protrude from the dendritic tips of sensory neurons, and are environmentally released through a cuticle pore. We have demonstrated that two EV cargoes, polycystin TRP channel PKD-2 and ion channel CLHM-1, are present in separate EV subpopulations. However, the mechanism by which EV cargoes are enriched into specific subsets is uncharacterized. We hypothesize that phosphoinositide asymmetry throughout the cilia affects how cargoes are selectively packaged. Despite making up only a small fraction of cellular phospholipids, phosphoinositides have essential functions in vesicular trafficking, ciliary localization of receptor proteins, ion channel regulation, and endocytic and exocytic processes. Phosphoinositides localize to discrete subcompartments of the ciliary membrane, with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) excluded from the cilium

proper. Regulatory enzymes may play a spatially-dependent role in maintaining the lipid asymmetry necessary for proper ciliary signaling. $PI(4,5)P_2$ is converted to PI(4)P by the phosphatase INPP-1, while PI(4)P is phosphorylated to $PI(4,5)P_2$ by the kinase PPK-1. We found that INPP-1::GFP preferentially localized to the cilium proper rather than the cilium base. Using Total Internal Reflection Fluorescence (TIRF) microscopy, we observed that *inpp-1(gk3262)* animals had increased release of PKD-2::GFP-containing EVs with no impact on the CLHM-1::tdT EV subpopulation. Conversely, overexpression of PPK-1 decreased CLHM-1::tdT EV release with no impact on PKD-2::GFP EVs. Our results imply that differences in INPP-1 and PPK-1 localization could lead to enrichment of specific phosphoinositide species in ciliary subcompartments in order to regulate the release of distinct EV subpopulations.

306 **The conserved kinase NEKL-4/NEK10 orchestrates ciliary integrity and mitochondrial dynamics to modulate hyperglutamylation-induced neurodegeneration** Kaiden M Power[1], Adriele Silva[2], Shaneen Singh[2], Ken Nguyen[3], David Hall[3], Christopher Rongo[4], Maureen Barr[1] [1]Genetics, Rutgers University, [2]The City University of New York, [3]Albert Einstein College of Medicine, [4]Microbiology, Rutgers University

Neurodegenerative diseases present a severe and significant medical concern. The health of neurons depends in large part on mitochondrial localization and metabolic function. Microtubule-based transport is important for proper mitochondrial localization and function, and thus to neuron health. Consequently, proteins that affect microtubule stability play indirect yet important roles in the regulation of mitochondria function. A loss-of-function mutation of the deglutamylase CCP1 causes infantile-onset neurodegeneration and leads to excessive glutamylation of microtubules and defects in transport of mitochondria in neurons. CCP1 is evolutionarily conserved. *C. elegans ccpp-1* functions in ciliated sensory neurons where it affects microtubule stability of neuronal cilia. We performed a forward genetic screen to identify suppressors of *ccpp-1* age-related ciliary degradation. We identified a mutation in the NIMA-related kinase *nekl-4*, an ortholog of mammalian NEK10. NEK10 is associated with both cilia dysfunction and disrupted mitochondrial homeostasis in mammalian cells. We found that NEKL-4 acted as a negative regulator of ciliary stability independent of microtubule glutamylation and via an unknown mechanism.

To uncover the NEKL-4 mechanism of action and to determine the role of NEKL-4 and CCPP-1 in mitochondrial dynamics, we generated CRISPR-tagged fluorescent wild type, overexpressed, and kinase dead (KD) forms of NEKL-4. Wild-type and NEKL-4(KD) did not localize to cilia, but were associated with mitochondria. Time lapse microscopy showed that NEKL-4 and mitochondria are co-transported, which suggests that NEKL-4 may regulate mitochondria transport in the ciliated neurons. Additionally, our imaging showed that both *nekl-4* and *ccpp-1* affect mitochondrial morphology, localization, and oxidation state in these neurons, all of which may impact cilia structure. In *ccpp-1* mutants, NEKL-4(KD) translocated to cilia and suppressed ciliary degeneration. Our preliminary transmission electron microscopy analysis indicates that *nekl-4(KD)* mutants display abnormal ciliary ultrastructure, consistent with a role in microtubule stability. These results suggest that NEKL-4 mediates communication between cilia and mitochondria. Our work adds to a growing body of evidence suggesting a connection between cilia stability and mitochondrial function that, when perturbed, could instigate neuronal degeneration.

307 **Analysis of hundreds of genes with limited functional annotation that are required for normal mammalian development** David Beier[1,2], Shamil Sunyaev[3] [1]Seattle Children's Research Institute, [2]University of Washington, [3]Harvard Medical School

Genes required for human development are often haploinsufficient when heterozygous for the null allele. Using an empirical Bayes method, we estimated selection coefficients (s_{het}) for heterozygous protein truncating variants (Cassa et al., Nat Genet 2017). Other methods have been proposed (Agarwal et al., eLife 2023; Zeng et al., bioRxiv 2023) and there are powerful heuristics to assess selective constraint (Karczewski et al., Nature 2020). We found that genes under the strongest selection were enriched for autosomal dominant disease genes, embryonic lethal mouse knockouts, and known developmental signaling pathways. Re-analysis using an updated s_{het} algorithm (Seplyarskiy et al., bioRxiv 2022) of 9000 protein interactions and 6000+ mouse knockouts generated by KOMP confirms these results.

Remarkably, despite the evident importance of the highly selected genes, there is a relative lack of functional annotation. **Less than 20% of the genes in the top s_{het} decile have a human disease association.** Using a Pubmed citation metric, 2/3 of this decile have less than the average for all genes. That the functions of most genes in this cohort are not known is astonishing.

While the case for studying loci under strong selection is compelling, the task of high-throughput functional analysis is daunting, especially for genes that affect patterning and organogenesis (vs. those with cell-autonomous effects). Our efforts at whole-embryo mouse analyses have been challenging, mainly due to the task of husbandry and sample collection. Novel methods of both *in vitro* and *in vivo* perturb-seq may be useful, but these are also resource-intensive. As such, it is attractive to first pursue bioinformatic analyses to infer gene function, which is facilitated by the large amount of expression and proteomic

data now available.

We are therefore developing a ranked list of 1000 genes of limited functional annotation for which we have evidence of strong selection, using a variety of methods. Genes in this list are ranked based on similarity to known key players in human development. As features, we have combined several existing selection metrics applied across major population sequencing datasets and have amended human population genetics considerations with a new comparative approach that focuses on gene losses in the mammalian phylogeny. Next, we will use domain composition, localization signals, expression patterns and protein interaction networks to prioritize genes. This will enable the generation of functional hypotheses that can then be validated experimentally.

308 **Signatures of selection associated with haploid biased transcript exchange in murine round spermatids** Daniel E Shaw, Jeffrey M Good University of Montana

Genomic conflicts are assumed to be pervasive drivers of genome evolution, yet there is limited evidence linking rapid molecular evolution with mechanistically plausible drivers of conflict. Mammalian spermatogenesis includes a prolonged period of post-meiotic development which introduces the potential for rapid evolution driven by haploid-level selection. However, early post=meiotic cells are effectively diploid because they share transcripts through cytoplasmic bridges. Some transcript sharing is essential for proper sperm development, but it also introduces the potential for the evolution of selfish elements that evade sharing. A recent mouse single-cell expression study found extensive allelic skew in the degree to which post-meiotic genes share expressed transcripts, raising the potential for widespread genetic conflict and haploid selection at this critical stage of development. Here, we combine a comparative genomic analysis of protein-coding, gene expression, and non-coding evolution to identify signatures of selection over deep evolutionary timescales in rodents. First, we compared the mouse and rat genomes and found extensive divergence in 3' UTR of genes found to have an allelic bias in haploid transcriptomes compared to genes with equal transcript exchange. This supports the role of the UTR as a site targeted by cellular transport machinery. Second, we compared whole exome sequences from 38 murine rodents spanning the divergence between mouse and rat (~15 million years) to identify genes under pervasive positive selection across spermatogenesis and for skewed genes in house mice. We found 279 genes with skewed transcript bias evolving under positive selection across rodents. However, this was not a significantly enriched amount compared to genes with equal exchange. We found that skewed genes under positive selection were associated with sperm motility. Finally, we utilized RNA-seq data from house mice to identify co-expression modules enriched for biased transcript exchange. We found that these modules tended to be mis-regulated in hybrid crosses potentially reflecting disruption of transcript exchange as a consequence of evolutionary divergence. This suggests that mis-regulated transcript exchange between hybrid spermatids may cause intra-genomic conflicts and contribute to reproductive isolation. Together, this work supports a model of rapid molecular evolution of biased transcript exchange as a driver of genomic conflict.

309 **Quantitative Trait Locus mapping in a mouse model of ischemic stroke identifies Macrophage Scavenger Receptor 1 (*Msr1*) as modifier of cerebral infarct volume** Douglas A Marchuk[1], David L Aylor[2], Han Kyu Lee[1] [1]Molecular Genetics and Microbiology, Duke University, [2]Biological Sciences, North Carolina State University

Ischemic stroke is caused by a disruption of the blood supply to the brain leading to neuronal cell death. Genetic studies of ischemic stroke have identified numerous gene variants that increase the risk to develop stroke. In stark contrast, genetic studies of stroke outcomes, such as the infarct territory size, are confounded by many uncontrollable variables, leading to a paucity of gene targets for treatment of an incipient stroke. Using genetically diverse inbred strains of mice and a surgically induced model of ischemic stroke, we used quantitative trait locus mapping to identify novel gene targets modulating infarct size, which varies greatly across inbred strains. Although infarct size is largely determined by the extent of collateral vessel connection between arteries in the brain that enables reperfusion of the ischemic territory, we have identified strain pairs that do not vary in this phenotype, but which nonetheless exhibit large differences in infarct size. In this study we performed QTL mapping in mice from an intercross between two such strains, WSB/EiJ and C57BL/6J. We identified a strong locus on chromosome 8 that overlaps with a locus of similar direction and effect previously mapped in an intercross between C3H/HeJ and C57BL/6J strains. To identify causative genes within the overlapping genetic interval, we surveyed nonsynonymous coding SNPs and performed RNA sequencing data analysis for all three mapping strains. We identified macrophage scavenger receptor 1 (*Msr1*) as a candidate gene that harbors multiple coding SNPs predicted to be damaging. Using *Msr1*-deficient mice, we demonstrate that cerebral infarct volume after stroke induction is increased in a strain background where reperfusion effects due to collateral vessels is blunted. The identification of neuroprotective genes such as *Msr1* provides new genes for future mechanistic studies of infarction following ischemic stroke and provides novel gene/protein targets for therapeutic development.

310 **The genetic regulation of dose response in a diverse mouse cell population is revealed by cell morphology QTL (cmQTL)** Callan O›Connor[1], Gregory R Keele[1,2], Whitney Martin[1], Daniel Gatti[1], Brian R. Hoffmann[1], Ron Korstanje[1], Gary

Churchill[1], Laura Reinholdt[1] [1]The Jackson Laboratory, [2]RTI International

Genetically diverse cell populations derived from mice are powerful tools for studying gene-environment interactions. New approach methodologies such as these are of increasing interest for modeling population variability as they offer genetic diversity and potentially, predictive value for focused *in vivo* studies in extreme genotypes. Using fibroblasts derived from a laboratory mouse genetic reference population (Diversity Outbred population), we performed high-content imaging to capture hundreds of cell morphology traits during exposure to the oxidative stress and DNA damage-inducing arsenic metabolite monomethylarsonous acid (MMA^{III}). We applied dose-response modeling to capture latent parameters of dose-dependent traits (i.e. EC50) and then used these parameters to map several hundred cell morphology quantitative trait loci (cmQTL). We found that cmQTL include genes that are known to be associated with cellular response to arsenic exposure, including *Abcc4* and *Txnrd1,* as well as novel gene candidates like *Xrcc2*. We also found significant variation in baseline traits, demonstrating that genetic variation influences basic aspects of cell and nuclear morphology in culture. We show that cmQTL are reproducible, that their allele effects predict response, and that they point to key molecular initiating events that are genetically regulated. This study provides a proof-of-principle for using genetically diverse mouse cell lines in toxicology screens to identify population level responses and suggest mechanisms underlying intrinsic variation in cellular response to chemical exposure.

311 **QTL mapping reveals independent loci affecting the magnitude and composition of inactivated influenza vaccine- and adjuvant- mediated immune responses in an F2 intercross** Marta C Cruz-Cisneros[1,2], Timothy A Bell[1], Pablo Hock[1], Colton L Linnertz[1], Kelsey E Noll[3], Breantie Parotti[1], Fernando Pardo-Maneul de Villena[1,4], Mark T Heise[1,3,4], Martin Ferris[1] [1]Department of Genetics, University of North Carolina, Chapel Hill, [2]Curriculum in Genetics and Molecular Biology, UNC Chapel Hill, [3]Department of Microbiology, University of North Carolina, Chapel Hill, [4]Lineberger Comprehensive Cancer Center, UNC Chapel Hill

influenza infection. However there is still significant variation between individuals in their protection against influenza after immunization, even despite the inclusion of adjuvants which boost immune responses. Better understanding of the genetic factors that influence these intra-individual differences would provide data for next-generation vaccine and adjuvant development. We screened several strains of Collaborative Cross (CC) mice with both an unadjuvanted inactivated influenza (iFlu), and iFlu adjuvanted with alum. We observed significant and differential heritability in the magnitude and composition of the antibody responses across these two treatments. We chose to generate a large F2 intercross between CC002/Unc and CC072/TauUnc. CC002 showed a robust response to iFlu, that was not significantly improved by alum adjuvanting. In contrast, CC072 showed a muted response to iFlu, but had a robust increase in overall antibody levels following alum adjuvanting. We assigned F2 mice into two arms, as in our initial experiment (iFlu immunization alone, and iFlu immunization adjuvanted with alum). Based on the phenotypic variation in this F2 population, we have identified six quantitative trait loci (QTL) contributing to different aspects of these variable immune responses. These include four loci that only have an effect on antibody responses with unadjuvanted iFlu, one locus that only has an effect following alum-adjuvanted iFlu, and one locus that has an effect in both treatment groups. Deconvolution of the mechanisms underlying these loci will help identify pathways critical to understanding and developing novel immunization and adjuvanting strategies.

312 **Characterizing the influence of genetic variation on cell states** Selcan Aydin[1], Daniel A Skelly[1], Ted Choi[2], Laura G Reinholdt[1], Christopher L. Baker[1], Gary Churchill[1], Steven C Munger[1] [1]The Jackson Laboratory, [2]Predictive Biology, Inc.

Genetic variation leads to phenotypic variability in pluripotent stem cells which limits their use in regenerative medicine. Recent studies have investigated the impact of genetic variation on pluripotency maintenance and differentiation capacity. However, our understanding of how differences in the pluripotent state influence gene regulation in later stages of development is limited. In this study, we present a genetic characterization of neural progenitor cell (NPC) lines that are differentiated from embryonic stem cell (ESC) lines derived from genetically diverse Diversity Outbred (DO) mice. We have measured the expression of more than 14,000 genes in 186 DO NPC lines. For 127 of these DO lines, we quantitated over 12,000 genes in both genetically identical ESC and NPCs. Gene expression showed variable agreement between the two cell states. Neural development-related genes were more abundant, while genes involved in pluripotency maintenance had lower abundance in NPCs compared to ESCs. Genetic diversity significantly influenced transcript abundance in NPCs, with over 90% of measured transcripts exhibiting non-zero heritability. Genetic mapping identified 4,060 expression quantitative trait loci (eQTL) at significance in NPCs, one-third of which were also observed in ESCs with concordant allele effects. The remaining two-thirds of eQTL were unique to NPCs and were overrepresented for genes involved in cell-cell signaling and nervous system development. Three distant eQTL hotspots, regions of the genome modulating the expression of many genes and likely harboring essential transcriptional regulators, were uniquely observed in NPCs, on Chromosomes 1, 10, and 11. Targets of the hotspot on Chromosome 1 were overrepresented for genes involved in mRNA processing, chromosome organization, and the cell cycle, particularly the spindle assembly checkpoint (SAC) and related processes. Notably, mediation

analysis identified *Pign* expression in the ESC state as the top candidate regulator. Recent work has implicated *Pign* as a crucial regulator of the SAC where aberrant *Pign* expression leads to SAC dysfunction and chromosomal missegregation. These findings suggest that genetic variation influencing gene expression in an early developmental cell state (ESC) can impact gene regulation in a more advanced developmental cell state (NPC).

313 **Leveraging Mammalian Genetics to Silence Infectious Diseases** Clare M. Smith Molecular Genetics and Microbiology, Duke University

A key feature of biological systems is genetic diversity. The fundamental importance of this diversity in evolutionary processes cannot be overstated: it is essential for population survival against challenges such as environmental change or attack by novel pathogens. This genetic diversity also presents extraordinary difficulties for infectious disease researchers defining the mechanisms of host and pathogen interactions that underly disease. Dissecting mechanisms at the molecular level is especially difficult when diversity is ignored and circumvented by focusing on genetically uniform populations, such as single inbred mouse lines. Despite some success, these approaches cannot address important biological and medical problems in which genetic diversity is most probably the fundamental element. For example, the outcome of infection with *Mycobacterium tuberculosis* (*Mtb*) varies dramatically between individuals; ranging from asymptomatic infection, to active and lethal disease. While the basis for these heterogeneous reactions is poorly understood, there is significant evidence that genetic diversity is a major underlying element. In the Smith Lab, we approach this problem by leveraging new mammalian and bacterial genetic resources to study each of these variables in isolation and in combination. Host diversity is modeled using mammalian genetic panels including the BXD, Collaborative Cross (CC) and Diversity outbred (DO) panels. Bacterial variation is concurrently incorporated using new genetic reporters or libraries of *M. tuberculosis* mutants (TnSeq). Using this highly tractable "dual genome" system, our research program focuses on defining the mechanisms by which genetic variation in the host and pathogen impact interventions and outcomes to tuberculosis in model systems and human patients. By combining both host and bacterial genetics approaches, we are defining the host pathways that mediate protection, and the bacterial tactics used to exploit and evade them.

314 **Natural partial loss of function variants in *SEL1L* rescue NGLY1 deficiency by altering ERAD and improving proteasome function** Travis Tu›ifua, Clement K Chow Human Genetics, University of Utah

N-glycanase 1 (NGLY1) deficiency is a rare disease caused by autosomal recessive loss of function mutations in the *NGLY1* gene. Patients suffer from movement disorder, developmental delay, liver dysfunction, and alacrima. NGLY1 removes N-linked glycans from glycoproteins in the cytoplasm and is thought to help clear misfolded glycoproteins from the endoplasmic reticulum (ER) through the ER associated degradation (ERAD) pathway. Despite this, the physiological significance of NGLY1 in ERAD is not understood. The best characterized substrate of NGLY1 is NRF1, a transcription factor that upregulates proteasome expression and the proteasome bounce back response.

Our lab created a *Drosophila* model of NGLY1 deficiency that faithfully recapitulates disease phenotypes in patients, including movement disorder, seizures, and lethality. We performed a *Drosophila* modifier screen using this model of NGLY1 deficiency and identified a number of modifiers that reduce the lethality of the model. In particular, we identified two putative loss of function variants in *SEL1L*: S780P and Δ806-809. Both variants are localized in the SEL1L cytoplasmic tail, an uncharacterized domain of the protein. SEL1L is a component of the ERAD complex that retrotranslocates misfolded proteins from the ER to the cytoplasm for degradation.

To test the interaction between these *SEL1L* variants and *NGLY1*, we created CRISPR mutant fly lines that carry these *SEL1L* variants in a common genetic background and tested them with our model of NGLY1 deficiency. We determined that these variants are partial loss of function. Validating our screen, the $SEL1L^{P780}$ and $SEL1L^{\Delta 806\text{-}809}$ variants increase the survival of the NGLY1 deficiency model, compared to the $SEL1L^{S780}$ variant. Further, we found that the $SEL1L^{P780}$ and $SEL1L^{\Delta 806\text{-}809}$ variants are protective against proteasome inhibition, leading to increased survival, in heterozygous *NGLY1* null flies. We also find that these variants modify general ERAD function. We hypothesize that these SEL1L variants modify NGLY1 deficiency through NRF1 signaling and ERAD. These results will provide new insights into the role of NGLY1 in ERAD and the etiology of NGLY1 deficiency. SEL1L is a strong candidate modifier gene in patients, where variability in presentation is common.

315 **The *Drosophila melanogaster* enzyme Glycerol-3-phosphate dehydrogenase (GPDH1) interacts with Target of rapamycin (Tor) to control larval growth.** Shefali A Shefali, Madhulika Rai, Jason M Tennessen Biology, Indiana University Bloomington

Animal development requires that nutritional cues are properly integrated with signaling pathways and gene expression networks. Thus, nutrient-sensing proteins play an essential role in coordinating nutrient availability with growth and maturation. Although substantial work has been conducted on the endocrine regulation of carbohydrate metabolism,

questions remain about how changes in glycolysis, itself, is communicated across tissues. Here we investigate this question using the fruit fly, *Drosophila melanogaster*, which is ideally suited for studying interorgan communication. Our preliminary studies reveal that glycerol-3-phosphate dehydrogenase 1 (GPDH1), a key enzyme that regulates abundance of the glycolytic intermediate dihydroxyacetone phosphate (DHAP), interacts with the nutrient sensor Target of rapamycin (Tor) in the larval fat body to control brain growth. Specifically, when Tor activity is inhibited within the fat body of *Gpdh1* mutant larvae, the brain experiences ectopic growth and ultimately becomes tumorous. We have also determined that this overgrowth phenotype is associated with excess cell proliferation within the brain optic lobe. As an extension of these studies, we have also determined that Tor and GPDH1 act in a negative feedback loop, where Tor inhibition in the fat-body leads to increased GPDH1 expression, which depletes the DHAP pool and further suppresses Tor signaling. Overall, our studies demonstrate how changes in glycolytic flux affect growth signaling and hold the potential to uncover a fundamental mechanism by which GPDH1 and Tor non-autonomously regulate tissue growth in response to changes in carbohydrate metabolism.

316 **Time Flies, how? Unlocking the Secrets of Aging** Hongjie Li Baylor College of Medicine

Aging phenotypes have been observed and described for centuries and a number of different aging hypotheses have been proposed. However, several critical questions remain largely unaddressed in complex organisms. For example, do different cell types age at different rates? If yes, which cell types age the fastest across the whole body?

Here I will present our recent work on the Aging Fly Cell Atlas, a single-nucleus transcriptomic map of the whole aging *Drosophila*. We characterize 163 distinct cell types and perform an in-depth analysis of changes in tissue cell composition, gene expression, and cell identities. We further develop aging clock models to predict the fly age. Combining all aging features, we find unique cell type-specific aging patterns and could quantitatively rank the aging rate for all types. This atlas provides a valuable resource for studying fundamental principles of aging in complex organisms. Based on this platform, we could now evaluate how pro-aging and pro-longevity mechanisms impact different cell types across the entire fly. In addition to Aging Fly Cell Atlas, I will also share one unpublished work on how lifespan-extending treatment affects the fly aging at cellular resolution.

317 **Extracellular vesicles mediate venom protein function in the parasitoid wasp *Ganaspis hookeri*** Nathan T. Mortimer[1,2,3], Nicholas M. Bretz[1,3], Chris Lark[3], Jeremy D. Driskell[4] [1]Department of Biochemistry & Biophysics, Oregon State University, [2]Linus Pauling Institute, Oregon State University, [3]School of Biological Sciences, Illinois State University, [4]Department of Chemistry, Illinois State University

Organisms frequently encounter potential pathogens in their environments, and understanding the molecular mechanisms that underlie host-pathogen interactions can shed light on immunity and cell signaling. *Drosophila melanogaster* larvae are commonly infected by parasitoid wasps, and this *Drosophila*-parasitoid interaction serves as a model system to better understand immunity and host-pathogen relationships. In this system, infected flies mount a robust cellular immune response to infection. To overcome host immunity parasitoids have evolved venom proteins which target specific host immune mechanisms, along with host metabolism and development, and is transferred directly into the larval hemocoel during infection. The venom from the parasitoid wasp species *Ganaspis hookeri* blocks immune cell activation in infected hosts, thereby inhibiting the ability of the host to mount an effective immune response. This venom activity is mediated by a unique venom-specific isoform of the SERCA (Sarco/endoplasmic reticulum Ca^{2+}-ATPase) calcium pump (vSERCA). Immune cell activation is dependent on a calcium burst normally mounted by hemocytes following infection, and vSERCA activity counters the intracellular rise in calcium levels, rendering infected larvae unable to mount an effective response against the wasp egg. vSERCA is a multipass transmembrane protein and so is unlikely to be stable in the aqueous environment of the venom. Instead, we find that vSERCA and other venom proteins are packaged into venom-specific extracellular vesicles. Our biophysical analysis, using ultracentrifugation, nanoparticle tracking analysis, dynamic light scattering, and tunable resistive pulse sensing suggests the presence of distinct vesicle classes in *G. hookeri* venom. Identification of protein content of purified vesicles further supports the idea that venom proteins including vSERCA can be stored as cargo within venom vesicles. We hypothesize that these vesicles provide a delivery mechanism to mediate transport of cargo into host immune cells, and we find that vesicle disruption alters venom activity in *ex vivo* calcium assays. This vesicle-mediated packaging and delivery machinery represents an important parasitoid virulence mechanism, and understanding this mechanism will provide insight into host-parasite interactions, and possible applied uses in delivery of therapeutic agents or biopesticides.

318 **A *Drosophila* model to investigate cell-intrinsic and non- autonomous toxicity of C9orf72 familial ALS and FTD** Isabel Hubbard[1], Josh Dubnau[1,2] [1]Neurobiology and Behavior, Stony Brook University, [2]Anesthesiology, Renaissance School of Medicine, Stony Brook University

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases that lead

to severe cognitive and motor defects in patients. Both feature aggregation of the protein TDP-43. Non-cell autonomous effects are a hallmark of these disorders, which we have previously reported to be mediated at least in part by endogenous retroviruses. The most common genetic cause of both familial ALS and FTD is a hexanucleotide repeat sequence consisting of four guanine and two cytosine nucleotides (G_4C_2) in the first intron of the gene C9orf72, which can produce expansions of up to hundreds to thousands of repeats in patients. The C9orf72 repeat expansion is bidirectionally transcribed into sense and anti-sense RNA foci. Additionally, a non-canonical translation mechanism known as repeat-associated non-ATG translation (RAN translation) allows the expansion to be translated into five different dipeptide repeats (DPRs): GA, GP, GR, PA, and PR. Previous work has shown that multiple forms of the repeat cause premature death and neurodegenerative effects in animal models, including *Drosophila,* when expressed neuronally, with the arginine-rich DPRs producing the highest level of toxicity. Effects on glia, impacts on non-cell autonomous toxicity, and on activation of retrotransposons by the DPRs and RNA repeats respectively are more poorly understood. Here, we use glial cell type-specific expression of individual DPRs, of RNA repeat-only, or of the "pure" G_4C_2 repeat that is capable of producing both DPRs and RNA repeats, to systematically investigate both cell-intrinsic and non-cell autonomous toxicity of each of these components. With pan-glial expression, the pure G_4C_2 repeat and GR dipeptide are equally the most toxic forms of the repeat; but when focally expressed in SPG, the pure G_4C_2 repeat is more toxic than is the GR dipeptide. Imaging experiments reveal that while GR is the most toxic C9 transgene in the cells that it is expressed in, the pure G_4C_2 repeat causes the greatest degree of non-cell autonomous toxicity to other glial subtypes and neurons. The G_4C_2 and GR repeats, namely those capable of producing the GR DPR, also each triggered replication of the fly endogenous retrovirus mdg4, which has been shown to mediate non-cell autonomy in TDP-43 fly models. Experiments to untangle the differences in non-cell autonomy between the pure repeat and GR flies, and how mdg4 activation might be involved, are currently in progress.

319 **Yolk protein 3 (YP3) has both metabolic and immune functions in *Drosophila melanogaster.*** Krystal Maya-Maldonado, Amber Chou, Natania Kurien, Nichole A. Broderick Biology, Johns Hopkins University

The *Drosophila* YP3 protein is one of three yolk proteins involved in vitellogenesis; a process in which yolk accumulates in the oocyte to provide energy for maturation. Classically, the function of YP3 has been focused on reproduction. However, our evidence suggests it also has functions in immunity and metabolism. Previous transcriptional and proteomics studies in *Drosophila*, indicate that YP3 is altered in response to both the microbiome and during systemic infection. Comparing the YP3 protein sequence to the Conserved Domain Database, we identified conserved domains related to lipid metabolism, including a lipase conserved domain, an alpha/beta hydrolase domain, and a Triacylglycerol lipase domain. Triacylglycerol (TAG) is the most important caloric source and in *Drosophila*, TAG metabolism is crucial for several physiological processes. As such, TAG represents an important reservoir to survive stress conditions such as starvation or food deprivation. Using a loss of function YP3 mutant fly line (ΔYP3) we conducted starvation resistance assays and found that in presence of the microbiome, ΔYP3 female flies are slightly less sensitive than control flies to starvation. This difference is greatly accentuated in germ free (axenic) conditions. While control female flies are less sensitive to starvation than control flies with a microbiome, we observe that ΔYP3 germ-free females are significantly more sensitive to starvation. This suggest that YP3 has a role in the energetic status of the host that is modified by the presence of the microbiome. We next looked for potential protein motifs and domains in the YP3 protein sequence involved in immunity. We conducted a protein structure prediction and found an antimicrobial structure related to lysin proteins of phages. Such lysin proteins are peptidoglycan hydrolases that degrade the peptidoglycan layer causing bacterial lysis of both Gram-positive and Gram-negative bacteria. YP3 mutant female flies (ΔYP3) systemically infected with the Gram-negative bacterium, *Pectobacterium carotovorum (Ecc15)* exhibited higher pathogen load than controls, suggesting a role for YP3 in pathogen clearance during systemic infection. Considering our evidence, we propose that YP3 exerts both metabolic and immunity functions in the host.

320 **Tau functions in glia lipid droplet formation and protects against neuronal ROS with implications in disease progression.** Lindsey D Goodman[1], Isha Ralhan[2], Ye-Jin D Park[1], Matthew J Moulton[1], Oguz D Kanca[1], Ziyaneh S Ghaderpour Taleghani[1], Kanae Ando[3], Maria Ioannou[2], Hugo J Bellen[1] [1]Baylor College of Medicine, [2]University of Alberta, [3]Tokyo Metropolitan University

Toxic reactive oxygen species (ROS) become aberrantly upregulated in Alzheimer's Disease (AD) and tauopathies, causing oxidative stress (OS). Connections between ROS and lipid metabolism came upon our discovery that glia form lipid droplets (LD) in response to neuronal OS. This involves the active production and export of peroxidated (O_2^-)-lipids from stressed neurons, the shuttling of these lipids to glia via apolipoproteins, endocytosis of the lipids into glia, and their incorporation into LD. LD-mediated breakdown of these O_2^--lipids effectively reduces ROS levels in both neurons and glia, protecting the cells from damage. Evidence is accumulating that defects in glial LD formation contributes to AD disease progression. Here, we considered connections with the important disease-associated protein, Tau. Using established tauopathy Drosophila models, we specifically overexpressed Tau in neurons or glia, finding that Tau overexpression in glia disrupts LD formation in response to neuronal ROS and leaves these cells vulnerable to damage. We further probed for a physiological function of

Tau in glial LD formation, developing a novel and robust loss-of-function (LOF) fly allele and improving upon shortcomings of previously published *tau* LOF alleles. This revealed that fly *tau* loss results in reduced motor abilities and lifespans which can be rescued by re-introducing *tau* in a dosage manner with one or two copies of a genomic rescue construct. Using glia-specific rescue of fly *tau* in our mutant flies and RNAi targeting fly *tau*, we observed that Tau loss in glia is a strong contributor to these phenotypes. Mechanistically, Tau is required for glia to properly form LD in response to neuronal ROS and loss of glial Tau leaves these cells vulnerable to ROS-induced damage. Other microtubule associated proteins, Futsch and Ensconsin, were also found to be important for glial LD formation, supporting that it is Tau's function at microtubules underlying its role in this process. Last, we found that the function of Tau in glial LD formation is conserved in mammals using primary rat neuron:astrocyte co-cultures. Overall, this study uncovers an important role of Tau in glial LD formation in response to O_2^--lipid production in neurons. As defects in glial LD formation are seen with both Tau loss and overexpression, this work reveals that Tau imbalance is associated with sensitivity to ROS and has numerous implications in Drosophila and disease research.

321 **Tumor cytokine-induced hepatic gluconeogenesis contributes to cancer cachexia: insights from full body single nuclei sequencing** Ying Liu[1,1], Ezequiel Dantas[2], Miriam Ferrer[3], Yifang Liu[4], Aram Comjean[1], Emma E Davidson[3], Yanhui Hu[1], Marcus D Goncalves[2], Tobias Janowitz[3], Norbert Perrimon[1,5] [1]Dept. of Genetics, Harvard Medical School, [2]Weill Cornell Medicine, [3]Cold Spring Harbor Laboratory, [4]Harvard Medical School, [5]Howard Hughes Medical Institute

A primary cause of death in cancer patients is cachexia, a wasting syndrome attributed to tumor-induced metabolic dysregulation. Despite the major impact of cachexia on the treatment, quality of life, and survival of cancer patients, relatively little is known about the underlying pathogenic mechanisms by which tumors influence host metabolic homeostasis. Here, through examining the full body transcriptome of flies with *yki*^{act} gut tumors at single nuclei resolution, we systemically analyze the tumor-induced reprograming of host metabolism. Interestingly, we observe an abnormally increased level of hepatic gluconeogenesis in these flies. Using Drosophila and murine models, we demonstrate that hepatic gluconeogenesis is stimulated by tumor-induced Upd3/IL-6/JAK-STAT signaling. Our data further indicate that locally elevated hepatic gluconeogenesis is a causative factor of systemic metabolic defects that lead to poor prognosis. Altogether, our study uncovers a conserved role for Upd3/IL-6/JAK-STAT signaling in inducing tumor-associated host metabolic dysregulation, and as such, provides insights into the pathogenesis of human IL-6 signaling in cancer cachexia.

322 **The conserved transcription factor Hmx/HMX1 establishes a positive feedback loop to specify blue-sensitive photoreceptor fate.** Joseph Bunker[1], Mhamed Bashir[1], Sydney Bailey[2], Brooke Cayting[2], Jens Rister[2] [1]Biology, Umass Boston, [2]Umass Boston

The function of the conserved Hippo pathway in regulating growth is well understood, but recent evidence suggests an additional key role in cell differentiation. The nexus of the pathway, the Warts/Wts kinase (LATS1/2 in mammals), controls growth by repressing the co-activator and oncoprotein Yorkie/Yki (YAP/TAZ) via phosphorylation. Unphosphorylated Yki activates genes that promote growth and activates its repressor Wts, resulting in a regulation of Yki activity via negative feedback. However, the pathway is rewired in the *Drosophila* retina to control differentiation of post-mitotic color photoreceptors (PRs): Yki represses *wts* at the transcriptional level, thereby establishing a positive feedback loop that allows the pathway to function as a bi-stable switch. Furthermore, Yki promotes the blue-sensitive Rhodopsin Rh5 and represses the green-sensitive Rh6. Thus, either Wts represses Yki, giving rise to green-Rh6 (Hippo ON) fate or Yki represses *wts*, giving rise to blue-Rh5 (Hippo OFF) fate. The mechanism that establishes the positive feedback loop for the binary color fate decision is not known.

Here we elucidate a novel role for the conserved Q50 homeodomain transcription factor (TF) H6-like homeobox (Hmx) in rewiring the Hippo pathway to establish the Yki positive feedback loop. Yki, in combination with the PR permissive factors Otd and Tj, activates *Hmx* exclusively in Rh5/Hippo OFF PRs. *Hmx* knockdown causes a loss of Rh5/Hippo OFF PRs, while ectopic Hmx causes their gain, indicating that Hmx is necessary and sufficient to specify Rh5/Hippo OFF fate. Through epistasis analyses we demonstrate that Hmx acts downstream of Yki to repress both *Rh6* and *wts.* Moreover, we performed a luciferase reporter assay to show that Hmx antagonizes a second Q50 homeodomain TF, Pph13, to repress the *Rh6* promoter *in vitro* through a conserved Q50 motif. Taken together, the convergence of active Yki with PR-specific permissive factors allows for the activation of Hmx, which in turn represses *Rh6* and *wts,* thereby mediating a positive feedback loop to give rise to robust Yki activation and Rh5 expression. Our findings identify Hmx as a novel repressor of the Hippo pathway in terminal PR differentiation and demonstrate how context-specific Hippo pathway interactions give rise to unique neuronal fates.

323 **Promoter-proximal pausing of RNA Polymerase II as a key regulator of morphogenetic furrow progression in Drosophila larval eye development** Bonnie M Weasner[1], Brandon Weasner[1], Justin Kumar[2] [1]Biology, Indiana University, [2]Indiana University

The foundation of development for any organism, tissue or cell is the precise spatio-temporal control of gene expression. As such, various molecular mechanisms exist to ensure this process is executed properly. A unique mechanism deployed in higher eukaryotes, including flies and humans, is promoter-proximal RNA polymerase II (Pol II) pausing. During this type of transcriptional regulation, Pol II in conjunction with the DSIF and NELF protein complexes is recruited to the gene promoter and begins elongation but will stall ~20-60bp downstream of the transcriptional start site (TSS). Paused Pol II can then be released by the P-TEFb complex thereby allowing for productive elongation and gene expression. It has been proposed that this method of transcriptional regulation allows for synchronous gene expression in dynamically developing tissues.

We recently discovered that Pol II pausing plays a key role during the development of the eye-antennal disc. We find disruption of pause release in cells ahead of the advancing morphogenetic furrow leads to severe disruption of patterning, photoreceptor development and in some cases complete ablation of the compound eye. Furthermore, we determined these defects are the result of a halt in furrow progression rather than a lack of furrow initiation. Using CUT&RUN techniques we have examined the binding profile of stalled and productively elongating Pol II in whole eye-antennal discs and as expected find many genes which are key to proper specification, patterning and differentiation of the eye disc are subject to Pol II pausing. We are now combining INTACT nuclei isolation and CUT&RUN techniques to fine tune our analyses to specific cell populations within the disc. Our goal is to form a more complete understanding of the extent to which Pol II pausing influences development of the Drosophila compound eye.

324 **The histone acetyltransferase Nejire is recruited to the genome by the pioneer factor Zelda and activates gene expression during the maternal-to-zygotic transition.** Audrey Marsh[1], Sergei Pirogov[2], Abby Ruffridge[1], George Hunt[2], Tyler Gibson[1], Katherine Hullin[1], Mattias Mannervik[2], Melissa Harrison[1] [1]University of Wisconsin-Madison, [2]The Wenner-Gren Institute

At fertilization, the new diploid genome carries signatures left by the sperm and the egg. These features are removed from the zygotic genome as it is reprogrammed to totipotent state. At this time, the zygotic genome is transcriptionally quiescent and is gradually activated by maternally encoded factors loaded into the egg. Zygotic genome activation (ZGA) is controlled by a specialized set of transcription factors, called pioneer factors, that promote chromatin accessibility. The pioneer factor Zelda (Zld) is an essential activator of zygotic transcription in *D. melanogaster*. Before ZGA, the chromatin is in a naïve state with nucleosomes largely devoid of post-translational modifications. Zld-bound sites, however, are enriched for histone acetylation, suggesting a histone acetyltransferase (HAT) is functioning with Zld to promote ZGA. We identified that Nejire (Nej), the fruit fly homolog of the HAT family p300/CBP, co-occupies 1,202 loci with Zld in the early embryo. Indeed, recruitment of Nej and histone acetylation at these sites requires Zld. Similarly, in tissue culture histone acetylation is increased at regions where Zld functions as a pioneer factor. To determine if Nej and acetylation are necessary for ZGA, we degraded Nej and found 786 downregulated genes. These data show that pioneer factor recruitment of a HAT promotes histone acetylation and ZGA. To provide temporal control over Nej inactivation, we developed an optogenetic system. As expected, inactivation decreased histone acetylation, but counterintuitively did not disrupt Nej chromatin occupancy. Thus, we used this tool to study the role of histone acetylation in ZGA. After assaying transcription upon light exposure, we identified only a subset of Nej-dependent genes decreased in expression. Thus, there are catalytic-dependent and independent function of Nej in ZGA. To further define the relationship between Zld, Nej, histone acetylation and chromatin accessibility, we used a drug to catalytically inhibit Nej in tissue culture. We found inhibition of histone acetylation did not change chromatin accessibility, separating Zld-mediated chromatin accessibility from acetylation. Preliminary data from the embryo similarly suggests that histone acetylation is dispensable for chromatin accessibility. Combined with data from vertebrates, our work supports a conserved mechanism for ZGA in which pioneer factors prime the genome for activation by recruiting HATs to *cis*-regulatory regions.

325 ***Drosophila* hnRNP M homolog Rumpelstiltskin promotes Homie barrier activity and regulates Polycomb-dependent 3D interactions** Savanna F. Lyda[1,2], Catherine E. McManus[1], Juan Manual Caravaca[1], Yang Chen[1], Dagyeong Yang[1,2], Elissa P. Lei[1] [1]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, [2]Biological Sciences, University of Maryland

Chromatin insulators are DNA-protein complexes that help maintain genome organization by creating boundaries that separate active and inactive chromatin and control promotor-enhancer interactions. In *Drosophila,* Homing insulator at eve (Homie) is an insulator sequence that can regulate interactions between *even-skipped* (*eve*), a Polycomb Group (PcG) regulated gene, and its cis-regulatory elements. Homie also acts as a barrier to prevent PcG repressive chromatin spreading into the neighboring essential *TER94* ubiquitously expressed gene. The mechanism behind Homie insulator activity and the requirement of trans-acting factors is unknown.

To this end, we determined that Homie barrier activity in all tissues tested relies on Rumpelstiltskin (Rump), as well as two chromatin insulator proteins, Centrosomal Protein 190kD (Cp190) and CCCTC-binding factor (CTCF). To address whether

chromatin compaction at the *eve-TER94* locus may be altered, we performed 3C analysis in Kc cells after depletion of Rump, Cp190, or CTCF. We found an increase in *cis*-looping within the PcG domain after Rump but not Cp190 or CTCF depletion. We also detected H3K27me3 spreading onto *TER94* in Rump-depleted cells and increased H3K27me3 levels at PcG domains genome-wide by ChIP-seq. To visualize potential changes in 3D genome organization after Rump depletion, we utilized Oligopaint DNA FISH to analyze pairwise PcG domain interactions in Kc cells. This analysis revealed that distances between distal PcG domains increase after Rump depletion. However, depletion of Cp190 or CTCF showed no effect, suggesting Rump regulates PcG through a mechanism unrelated to insulator regulation. We next performed immunofluorescence and ChIP-seq assays to detect Polycomb (Pc), a protein in the PRC1 complex, in Kc cells after Rump depletion. These data show global reduction of chromatin bound Pc after Rump depletion. Taken together, in Rump-depleted cells we observe increased compaction of the PcG domains *in cis* yet decreased compaction *in trans*. Future experiments aim to elucidate how Rump affects the localization of Pc to chromatin and influences reciprocal *cis* and *trans* PcG interactions. Through understanding these interactions, we seek to gain additional insight into how Rump promotes Homie barrier activity.

326 **Damage-Responsive enhancers activate the regeneration program by sensing sequential inputs** John Quinn, Robin Harris Arizona State University

In organisms that can regenerate, thousands of genes are upregulated following damage in order to regenerate missing tissue. Only recently has it been identified how these genes become activated following damage. Damage-Responsive (DR) enhancers are regeneration regulatory regions that recognize damage stimuli and respond by activating genes essential for regeneration. To study DR enhancer activity, our lab uses the wing imaginal disc, which is highly regenerative. Currently, we do not know how DR enhancers become activated following damage. My work focuses on identifying the damage-induced signals that activate DR enhancers to promote regeneration. To study regeneration, damage is caused by a genetic ablation system called DUAL control, consisting of a bipartite gene expression system that induces apoptosis in the disc while simultaneously activating the Gal4/UAS system in the surrounding regenerating cells to knockdown or overexpress any gene of interest. This system has been paired with GFP reporters of DR enhancers to visualize their activity during regeneration.

Using these techniques, it was found that the transcription factor of the JNK signaling pathway is necessary for enhancer activation following damage, but developmental instances of JNK expression is insufficient for enhancer activation. An important question that remains unresolved, is whether a damage-induced factor is needed along with JNK signaling, or is it differences in levels of JNK signaling that is required for enhancer activation. To narrow down potential candidates involved in DR activation, a damage stimulus was initiated while blocking cell death. It was found that DR is capable of being activated independent of cell death. This suggests that the activating factor is likely a direct downstream targets of JNK, one of those being JAK/STAT, which is known to be involved in regeneration. To test if the transcription factor for the JAK/STAT pathway is involved, a new DR transgenic reporter was created lacking the JAK/STAT transcription factor binding sites which reduced enhancer activation. JAK/STAT is not only required for full activation of DR but also controls the spatial domain where DR is expressed. Upon knockdown of the JAK/STAT pathway DR expression is limited to the cells directly adjacent to the wound.

Additionally, I have demonstrated that lower levels of JNK expression seen in a developmental context are insufficient for enhancer activation, and high levels of JNK expression that occur during damage are required for enhancer activation. My work has shown that both damaged-induced factors and levels of JNK signaling are essential for DR activation. Overall, my research aims to identify the minimal components necessary for DR activation, which might be used to activate DR in non-regenerative tissue to permit regeneration.

327 **Bicoid-nucleosome competition sets a concentration threshold for transcription** Eleanor Degen, Shelby Blythe Molecular Biosciences, Northwestern University

Concentration gradients of maternal transcription factors establish patterns of gene expression during a time in early embryonic development characterized by frequent mitotic divisions, rounds of genome replication, and chromatin reorganization. In the *Drosophila* embryo, an exponential gradient of the transcription factor Bicoid (Bcd) activates the earliest expressed patterning genes across the anterior-posterior axis. Bcd successfully navigates the chromatin of the replicating genome to bind its sites and facilitate transcription. However, we do not fully understand how genomic context leads to differential transcriptional outputs across Bcd concentrations. We aim to model how enhancer sequence, chromatin, and DNA replication together determine the Bcd concentration-sensitivity of the transcriptional process. By live-imaging an MS2-MCP transcriptional reporter for the Hunchback P2 (HbP2) enhancer, we have found that the length of the delay between mitosis and the initiation of transcription uniquely reflects Bcd concentration-sensitive regulation. We have defined a stochastic model of transcriptional regulation that accurately predicts transcriptional onset times using mathematical descriptions of Bcd-nucleosome competition for occupancy and the probability of DNA replication at HbP2. Our work suggests that Bcd's ability to outcompete nucleosomes dictates a Bcd concentration threshold for expression, while DNA replication limits the rate of

transcriptional activation at high concentrations where Bcd readily outcompetes nucleosomes. Disrupting nucleosome stability by promoting pioneer factor binding to HbP2 both expands the MS2 expression domain and preserves onset time variance at high concentrations, supporting these hypotheses. An understanding of the critical parameters of nucleosome positioning, stability, and replication dynamics should allow for genome-wide predictions of the concentration-sensitivity of Bcd binding events.

328 **Spatiotemporal regulation of *orthodenticle* during early embryogenesis and retinal development in *Drosophila*** Jungwon Lee, Campbell Berry, Sosina Abuhay, Grace Carey, Marjorie Wang, Rhea Datta Hamilton College

Activating and expressing the right gene, in the right tissue, at the right time is critical for proper development. Gene expression is carefully coordinated by direct binding events between cell-specific transcription factor proteins (TFs) and specific cis-regulatory elements (CREs). This two-part system regulates when, where, and at what levels the coding region of a corresponding gene is transcribed. Understanding the intrinsic mechanisms of TF-CRE interactions remains a primary challenge in developmental biology. Adding a layer of complexity to this is the fact that the same gene is often reused in multiple tissues. As a result, the development of different organ systems is contingent on the correct regulation of a shared gene which is regulated by modular CREs. The modularity built into CREs is a source of robust gene expression, but the mechanisms that dictate whether a CRE is redundant, reused, or redeployed remain poorly understood. Here we perform a detailed examination of CRE redundancy and pleiotropy at the highly conserved *otd* locus in *Drosophila* - which contains CREs that regulate the embryonic and retinal expression of *otd*. We identify multiple, redundant otd CREs that are active during embryogenesis and retinal development. We also identify TF binding sites that are enriched in embryonic CREs vs. retinal CREs, and examine whether these specific sequences inform spatiotemporal CRE activity. Finally, we aim to understand the role of chromatin accessibility at the regulatory loci of genes that are active in two developmental contexts.

329 **Integrator-mediated clustering of poised RNA polymerase II synchronizes histone transcription** Feiyue Lu[1], Brandon J Park[2], Rina Fujiwara[3], Jeremy E Wilusz[3], David S Gilmour[2], Ruth Lehmann[4,5], Timothee Lionnet[6,7,8] [1]Institute for Systems Genetics, NYU School of Medicine, [2]Center for Eukaryotic Gene Regulation, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, [3]Verna and Marrs McLean Department of Biochemistry and Molecular Pharmacology, Therapeutic Innovation Center, Baylor College of Medicine, [4]Whitehead Institute for Biomedical Research, [5]Department of Biology, Massachusetts Institute of Technology, [6]Institute for Systems Genetics, NYU Grossman School of Medicine, [7]Department of Cell Biology, NYU Grossman School of Medicine, [8]Department of Biomedical Engineering, NYU Tandon School of Engineering

Dynamic clustering of transcription players, including RNA polymerase II (Pol II), is thought to rely on multivalent interactions of their intrinsically disordered regions, thereby enhancing active transcription. Using the histone locus bodies (HLBs) of *Drosophila* nurse cells as a model, we find that Pol II forms long-lived, transcriptionally poised clusters distinct from liquid droplets, which contain unbound and paused Pol II. Depletion of the Integrator complex endonuclease module, but not its phosphatase module or pausing factors disperses these Pol II clusters, leaving HLBs intact. Consequently, histone transcription fails to reach peak levels during S-phase and aberrantly spills over throughout the cell cycle. We propose that clustering sequesters numerous poised Pol II molecules near gene promoters to ensure synchronous and efficient gene activation at desired times.

330 **Establishing the zebrafish as a model to study the pharynx** Kiyohito Taimatsu, John Prevedel, Aniket V Gore, Amit Puthan, Miranda Marvel, Daniel A Castranova, Madeleine Kenton, Gennady Margolin, Andrew Davis, Van Pham, Brant Weinstein NICHD, NIH

The pharynx and associated organs have important roles in breathing, eating, drinking, digesting, speaking and protecting the body from infection. Pharyngeal dysfunction can manifest as dysphagia, persistent palatal displacement, respiratory function decline, or exercise intolerance. Secondary complications are serious and life threatening and can include aspiration pneumonia, weight loss, and death. However, pharynx development is not well understood, in large part because of the inaccessibility of the developing pharynx to observation and experimental manipulation in most model organisms. I am establishing the zebrafish as a powerful model for observation and genetic and experimental analysis of pharynx development. Although the pharynx is more accessible in zebrafish than in mammalian models, it is still difficult to observe clearly because of its deep location and surrounding opaque tissues. I have developed powerful new tissue clearing methods customized for use with Hybridization Chain Reaction (HCR) to permit labeling and high-resolution imaging of pharyngeal tissues and cells even in older developing animals and adults. I have used these and other methods to describe developing and adult pharynx anatomy, and I have used scRNAseq to identify resident cell types of the pharynx and their gene expression signatures. My anatomical and molecular findings show the zebrafish pharynx is a complex structure with numerous specialized cell types, many of which correspond well to cell types identified in the mammalian pharynx. I have also used genetic screens to identify

pharynx-specific mutants that I am currently characterizing in detail. One mutant has more ionocytes and less mucous cells in the pharynx, suggesting a hidden balancing system between these cells, and demonstrating that genetic mutants can reveal new and unknown mechanisms in pharyngeal development. By developing new methods, uncovering mutants, and compiling fundamental information on pharyngeal anatomy and resident pharyngeal cell types, I am establishing the zebrafish as a valuable new model for experimental and genetic analysis of the pharynx.

331 **Dixdc1b is a novel downstream effector of Nckap1l (Hem1) during biliary system morphogenesis.** Kathryn M. Tuscano[1], Isabela M. Rivera Paz[1], Donavan S. O'Brien[1], Takuya Sakaguchi[2] [1]Cleveland Clinic Foundation, [2]Cleveland Clinic

Impaired formation of the biliary system contributes to cholestatic liver diseases. Cdk5 orchestrates branching morphogenesis of the intrahepatic biliary network by regulating its downstream effectors, including *nckap1l*, a Wave Regulatory Complex (WRC) component. We combined a forward genetic approach in zebrafish with unbiased computational quantification to identify a new mutation that genetically interacts with the *cdk5-nckap1l* pathway. The interacting mutation affects the *DIX Domain Containing 1b (dixdc1b)* gene, the homolog of a known substrate for Cdk5. *dixdc1b* mutant larvae show intrahepatic biliary network phenotypes similar to that in *nckap1l* mutant larvae. The genetic interaction between these two mutations is so strong that even double heterozygous larvae for *dixdc1b* and *nckap1l* consistently show a phenotype indistinguishable from *dixdc1b* homozygous mutant larvae, indicating that these two genes function in the same pathway. Overexpression of *dixdc1b* in *nckap1l* mutant larvae rescued all prominent intrahepatic biliary network phenotypes, including altered network branching pattern, actin dynamics, expression of WRC component proteins, and biliary system functions. In contrast, overexpression of *nckap1l* did not rescue the *dixdc1b* mutant phenotypes. These data together indicate that *dixdc1b* is a downstream effector of the *cdk5-nckap1l* pathway to regulate the branching morphogenesis of the intrahepatic biliary network. This study suggests that combined heterozygosity in components of this cascade may provide a genetic basis for some cases of cholestatic liver disease that may have been diagnosed as sporadic.

332 **Discovery of a new external organ in the zebrafish, an exciting model to study the immune response *in* and *ex vivo*** Daniel Castranova[1], Madeleine Kenton[1], Aurora Kraus[1], Jong Park[1], Marina Venero Galanternik[2], Gilseung Park[1], Daniel Lumbantobing[3], Miranda Marvel[1], James Iben[1], Louis Dye[1], Van Pham[1], Lucas Blevens[4], John K. Frazer[4], Brant M Weinstein[1] [1]NICHD, NIH, [2]University of Utah, [3]Smithsonian Institution, [4]University of Oklahoma

We recently discovered an external lobular structure on the outside of the adult zebrafish, just rostral to the gills and dorsal to the pectoral fin. The structure is only a few millimeters long, is not covered with scales or pigment cells, is transparent, and has a plexus of blood and lymphatic vessels on the inside. When removed, the lobe fully regenerates in a few weeks. The outer dermal layer of this lobe is teeming with immune cells, suggesting a role in immune surveillance. The lobe is easily removed and imaged and can be soaked in various antigens and other compounds to see how different cell types respond and interact. Interestingly, when lobes were soaked in fluorescently labeled SARS-CoV-2 spike protein, it was taken up by lymphatic vessels specifically, while lipopolysaccharide (bacterial) was taken up by goblet cells on the surface of the organ, and poly I:C (viral) was taken up by macrophages. Because it is external, transparent, and contains a variety of immune cells including T and B cells, neutrophils, and macrophages, and because zebrafish transgenic lines are available for high resolution imaging of immune, vascular, and other cell populations, this newly discovered tissue provides a superb model for *in vivo* and *ex vivo* imaging of the interaction between immune cells and their surrounding tissues, including blood and lymphatic vessels.

333 **Decoding Neutrophil Dynamics During Mycobacterial Infection Using Live Imaging, Single-Cell, and Dual Host-Pathogen RNA-Seq Approaches** Gopinath Viswanathan[1], Erika J Hughes[1,2], Ana María Xet-Mull[1], Eric M Walton[1], Graham Alexander[3], Devjanee Swain-Lenz[1,4], David M Tobin[5,6] [1]Molecular Genetics and Microbiology, Duke University, [2]University Program in Genetics and Genomics, Duke University, [3]Sequencing and Genomics Technologies Core Facility, Duke University, [4]3Sequencing and Genomics Technologies Core Facility, Duke University, [5]Molecular Genetics and Microbiolgy, Duke University, [6]Department of Immunology, Duke University

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a substantial global threat claiming the lives of around 1.6 million people worldwide in 2021. It stands as the second most deadly infectious disease after COVID-19, emphasizing the urgent need for proactive measures to address its impact. TB granulomas, the pathological hallmark of TB infection are known to harbor neutrophils, but their role during mycobacterial pathogenesis has not been fully explored. Upon infection with *Mycobacterium marinum* (*M. marinum*), adult zebrafish form mature granulomas with conserved characteristics of human TB granulomas. Here we use Myco-GEM, a granuloma explant system in zebrafish, to assess neutrophil dynamics and to directly manipulate neutrophil biology during mycobacterial infection. Using long-term high-resolution confocal imaging, we found active recruitment of neutrophils to the interface between the granuloma's necrotic core and its cellular layers. We observed heterogeneous populations of neutrophils with discrete behaviors in different microenvironments within the granuloma both via microscopy and through single-cell RNA-seq analysis of granuloma-associated neutrophils. We describe

strategies using this platform to manipulate neutrophil behavior within granulomas both pharmacologically and genetically, revealing their host-detrimental functions. By conducting bulk dual RNA-seq analysis on granulomas with dysfunctional neutrophils, we identified that specific neutrophil populations exert diverse effects on both host immune cells and the mycobacteria. They were found to exhibit immunoregulatory functions and additionally, could modulate the expression of the mycobacterial DevR regulon, a set of genes responsive to stress conditions such as hypoxia and nitric oxide thereby promoting mycobacterial survival within the granulomas. These findings provide new insight into the regulation of immune responses and the complex interplay between neutrophils and mycobacteria in granulomatous environments.

334 **A high-throughput whole-animal screen to identify modifiers of atherogenic lipoproteins** Daniel Kelpsch[1], Lishann Ingram[1], Liyun Zhang[2], Yuki Lin[1], Ikrak Jung[2], Stefan Harry[1], Mira Sohn[1], Thomas Lectka[1], Rexford Ahima[2], Jeff Mumm[2], Steven Farber[1] [1]Johns Hopkins University, [2]Johns Hopkins School of Medicine

Metabolic dysfunction is the leading cause of worldwide mortality and exhibits complex etiology. Increased apolipoprotein B (ApoB)-containing lipoproteins (B-lps) is a hallmark of cardiovascular and metabolic diseases. However, these B-lps transport lipids through the plasma to peripheral tissues and are essential to life. A single ApoB molecule decorates each B-lp and is functionally essential. However, the cellular mechanisms that regulate ApoB and B-lp production, secretion, transport, and degradation remain to be fully defined. Human B-lp biology is remarkably conserved in the zebrafish, making it the ideal system for identifying novel mechanisms of ApoB modulation. Therefore, the Farber lab generated an in vivo chemiluminescent reporter of ApoB that does not disrupt ApoB function. We performed a high-throughput chemiluminescence whole zebrafish screen of 3000 compounds and identified 48 ApoB-lowering compounds. One hit, enoxolone, is reported to inhibit Hepatocyte Nuclear Factor 4α (HNF4α), an orphaned nuclear hormone transcription factor known to regulate the expression of B-lp synthesis genes. Further, a loss of function HNF4α allele reduces lipoproteins during development, phenocopying the effect of enoxolone. We hypothesize that enoxolone modulates lipoprotein levels by inhibiting HNF4α, and are testing this using pharmaco-genetic interaction studies. The results of these studies will demonstrate the utility of our whole-animal high-throughput chemical screening approach. Cinnamon oil also reduced ApoB in our screen. Using liquid chromatography to fractionate this complex oil, we identified a single chemical, eugenol, that specifically reduces ApoB levels in whole animals and circulation. However, the mechanism of action remains unknown. Our future goal is to identify how eugenol affects B-lp levels in zebrafish and confirm this effect in a mammalian model. Ultimately, this research aims not only to identify novel ApoB-modulating therapeutics that would improve metabolic disease outcomes but also to provide fundamental insights into the regulation and function of ApoB.

335 **The atypical cadherin-encoding gene *cdh16* directs establishment and plasticity of acoustic startle response thresholds** Zackary Marshall, Susannah Schloss, Nicholas Santistevan, Jessica Nelson Cell and Developmental Biology, University of Colorado Denver

Properly tuned sensory thresholds enable animals to navigate complex environments. Sensory threshold establishment, maintenance, and plasticity are disrupted in a variety of human neurological disorders, including ASD, schizophrenia, and migraine. Although these processes are crucial for species' fitness and proper neurological functioning, the underlying mechanisms are not well understood. Through a forward genetic screen, we now establish the calcium-dependent adhesion protein (cadherin) encoding *cdh16* gene as a critical regulator of acoustic startle threshold establishment and plasticity. Compared to their wild type siblings, *cdh16* loss-of-function mutations result in hypersensitivity to low-intensity acoustic stimuli. Moreover, *cdh16* mutants fail to habituate to repeated stimuli, consistent with a role for this atypical cadherin in the dynamic regulation of sensory thresholds. Interestingly, although expression of *cdh16* has been observed in the brain and other cadherins are known to regulate multiple stages of nervous system development, our work provides the first evidence of a role for *cdh16* in the nervous system. To determine when *cdh16* might regulate acoustic startle threshold establishment and plasticity, we generated a heat-shock rescue transgene. Using this tool, we observed restoration of normal levels of acoustic responsiveness and habituation learning when we induced *cdh16* expression after the acoustic startle circuit is intact, suggesting that *cdh16* is likely not involved in early stages of neurodevelopment, but is required for later processes of circuit maturation and refinement. Finally, prior work indicates that *cdh16* plays a crucial role in the development of endocrine organs that maintain calcium homeostasis. In this context, *cdh16* functions together with Wnt/β-catenin signaling. To determine whether these pathways interact in the context of sensory processing, we applied the Gsk-3 antagonist XV to *cdh16* mutants and their wild type siblings. We found that while XV caused hyposensitivity to acoustic stimuli in wild type animals, it had no effect in siblings, consistent with a role for Wnt signaling in the expression or function of *cdh16.* Together our results establish a novel role for the atypical cadherin *cdh16* as well as Wnt/β-catenin signaling in the regulation of acoustic thresholds and provide a molecular foothold to understanding how sensory thresholds are tuned during development.

336 **PCP proteins guide the way to a fast escape** Joy H Meserve[1], Maria Navarro[2], Michael Granato[1] [1]Cell and Developmental Biology, University of Pennsylvania, [2]Neuroscience Graduate Group, University of Pennsylvania

As the brain develops, neurons must wire together to assemble functional circuits capable of directing diverse behaviors. While numerous molecular pathways that guide neurons to their synaptic partners have been identified, it is unclear if distinct neuron groups in a shared circuit employ overlapping pathways for coordinated connectivity. Guided by this query, we leveraged the simple and well-defined acoustic startle circuit in larval zebrafish to explore how the planar cell polarity (PCP) pathway orchestrates circuit assembly. The startle response is a protective behavior that is both evolutionarily conserved and clinically relevant as an indicator of broader nervous system function. Central to the zebrafish startle circuit are the Mauthner neurons, a pair of gigantic reticulospinal neurons that, in response to a startling noise, activate to initiate a rapid swim escape. We discovered that the PCP-associated atypical cadherins Celsr2 and Celsr3, as well as Celsr3's binding partner Frizzled 3a/Fzd3a, promote Mauthner axon guidance and growth. Celsr3 additionally directs axon guidance of excitatory spiral fiber neurons that synapse onto the Mauthner axon. These unique axo-axonic synapses are encapsulated by astrocyte-like glia, which mislocalize in *celsr3* mutants to ectopic spiral fiber axon projections, suggesting spiral fibers promote glial guidance. Our findings also reveal divergent functions for Celsr2 and Celsr3. *celsr2* single mutants display normal startle behavior and Mauthner axon development while *celsr3* single mutants display defects that are further enhanced in *celsr3;celsr2* double mutant larvae, resulting in Mauthner cell death. This indicates redundant and/or compensatory mechanisms ensure proper startle circuit development. Overall, our results reveal that Mauthner and spiral fiber neurons utilize a shared Celsr/Fzd molecular pathway to guide axons to their synaptic partners, a mechanism that may ensure coordinated assembly of the acoustic startle circuit.

337 **Functional characterization of human *G6PD* variants using a multiplexed assay in *S. cerevisiae*** Renee C Geck, Maitreya J Dunham Genome Sciences, University of Washington

Glucose-6-phosphate dehydrogenase (G6PD) deficiency affects over 500 million people. G6PD is important in red blood cells since it produces NADPH to detoxify reactive oxygen species, so individuals with G6PD deficiency may experience hemolysis after infections or exposure to oxidants, including many drugs. However, diagnosis by activity assays can give false negatives during hemolytic crises, and since *G6PD* is on the X chromosome carriers must be identified to avoid triggers during pregnancy. Thus accurate interpretation of *G6PD* genotype is valuable for preventing adverse drug reactions.

We connected *G6PD* genetic variants to their function using assays in *S. cerevisiae*, since human *G6PD* functionally complements the yeast homolog *ZWF1*. Yeast use NAPDH to produce methionine and antioxidants, so *zwf1Δ* yeast grow poorly in the absence of methionine and presence of hydrogen peroxide. Growth rate measurements and direct competition showed that *zwf1Δ* yeast expressing high-activity G6PD variants out-competed yeast with low-activity variants in this selective condition.

In order to scale our assay to address the unmet need of over 800 identified *G6PD* variants of uncertain effect, we created a barcoded variant library of nearly all possible single missense variants in *G6PD*. We transformed *zwf1Δ* yeast with this library and grew them in turbidostats without methionine and with hydrogen peroxide to select for G6PD activity. We took 10 samples from each population over 72 hours, which we deeply sequenced to calculate barcode frequency. Using long-read sequencing, we associated each barcode with a *G6PD* variant and used the change in frequency of associated barcodes over time to calculate a functional score for each variant.

We identified several regions of *G6PD* that are intolerant to mutation and confirmed that disrupting the dimerization domain reduces function. By benchmarking our assay with variants of known clinical effect, we will compute a level of evidence that can be integrated into variant interpretation frameworks.

In the future, we are conducting additional assays to measure the effects of *G6PD* variation on G6PD protein abundance and to determine the effects of multiple missense variants in one allele, since several *G6PD* variants identified in global populations affect two residues. Altogether, our study will enable clinical interpretation of a larger number of *G6PD* alleles and increase our understanding of how variation alters G6PD function.

338 **Identifying conserved aging regulators by high throughout lifespan screen in budding yeast** Weiwei Dang Huffington Center on Aging, Baylor College of Medicine

The budding yeast, Saccharomyces cerevisiae, is one of the most genetically tractable eukaryotic models. Yeast cells undergo asymmetric cell division, producing a smaller daughter cell, and have a finite replicative lifespan. This aging model has been quite fruitful in discovering the molecular mechanisms of the aging process. However, the replicative lifespan is traditionally measured by manually dissecting mother cells from daughter cells, which is a very tedious and inefficient process. Here, we present two high throughput methods for analyzing yeast replicative aging: a microfluidic-based high throughput lifespan assay and a population-based high throughput replicative lifespan screen. We show that the microfluidic-based high throughput lifespan assay provides consistent and reliable replicative lifespan measurements for yeast strains with an increase in efficiency

by 300-fold. Screening for mutant library for certain genetic traits has been a powerful approach to under basic biology. However, even with the microfluidic lifespan assay, looking for long-lived or short-lived mutants among thousands of strains remains a daunting task. Our population-based high throughput lifespan screen approach can identify long-lived and short-lived mutants from thousands of mutant strains simultaneously in a week-long experiment. These methods provide modern, reliable, and efficient ways for characterizing aging phenotypes for yeast mutant strains.

339 **Using evolutionary rate covariation to build protein-protein interaction networks and identify organelle crosstalk** Jordan Little[1], Nathan Clark[2] [1]University of Utah, [2]Biological Sciences, University of Pittsburgh

The physical organization of the cell is relatively well understood, and it is appreciated that there is functional similarity between proteins that occupy the same subcellular location, such as within protein complexes and organelles. Determining the subcellular location of proteins is relatively straightforward via traditional bench methods, but understanding functional interactions between proteins that do not share physical proximity requires a greater degree of prior knowledge to identify them. This is especially true for proteins that are only associated under specific conditions and for brief periods of time. However, functionally related proteins are under shared selective pressures, allowing us to utilize evolutionary methods to find these extremely transient relationships. Evolutionary rate covariation (ERC) is a method that leverages the rate of evolution for a protein as a proxy for selection and then finds pairwise proteins with correlated rates which would suggest shared evolutionary pressures and thus functions.

We have used a dataset of 343 yeast genomes, with 12,552 orthologous genes, to create a functional map of the yeast cell using ERC. Using the annotated subcellular location for yeast proteins we show that protein pairs with high ERC are not exclusively located within the same organelle. This observation indicates that ERC is able to identify proteins with functional relationships that do not co-localize, or that co-localize under very specific conditions that have not yet been observed. To further investigate, we built organelle specific protein-protein interaction networks, using ERC scores as the edges, to identify which organelles have the strongest evidence of connectivity and which specific proteins are driving that connection. To investigate the broader evolutionary conservation of the interactions we also calculated the ERC scores for all yeast-mammal orthologous genes using a dataset of 120 mammals and incorporated them into the edges of the network. This evolutionary protein-protein interaction network can be analyzed as fine-tuned as individual proteins pairs, all the way up to potential cross-talk between organelles, allowing us to build a functional map of the yeast cell in a way that is yet unattainable via experimental methods.

340 **Changing course: Regulation of Hexokinase 2 nuclear access and function** Mitch Lesko[1], Daksha Chandrashekarappa[2], Annette Chiang[1], Eric Jordahl[1], Katherine Oppenheimer[1], Chaowei Shang[1], Jacob Durrant[1], Martin Schmidt[2], Allyson O'Donnell[1] [1]Dept. of Biological Sciences, University of Pittsburgh, [2]Molecular and Microbial Genetics Department, University of Pittsburgh

Glucose is the preferred carbon source for most eukaryotes. Upon entry into the cell, glucose is rapidly converted to glucose-6-phosphate by hexo- or gluco-kinases. The yeast *Saccharomyces cerevisiae* (Sc) encodes three enzymes to phosphorylate glucose: Hxk1, Hxk2 and Glk1. Though predominantly cytosolic, in yeast and mammals isoforms of these enzymes can be found in the nucleus. This led to the suggestion of 'moonlighting' functions for hexokinases in this compartment, beyond their typical role in glucose metabolism. However, the molecular details underlying the activity of hexokinases, and other glycolytic enzymes, in the nucleus are often unclear. Past studies suggested that *Sc*Hxk2 entered the nucleus in glucose-replete conditions as part of a transcriptional complex that controlled glucose-repression of gene expression. In sharp contrast to this early model, our data demonstrate that Hxk2 accumulates in the nucleus in response to glucose starvation, and these data align well with the nuclear localization changes observed for mammalian hexokinases. Using high-resolution, quantitative fluorescence microscopy in live yeast cells, we define the conditions, *cis*-acting sequences, and trans-acting regulatory proteins needed for Hxk2 nuclear translocation. Our RNAseq analysis further demonstrates that Hxk2 has no major role in regulating glucose-repression of gene expression. These data demonstrate that Hxk2 does not moonlight as a nuclear transcription factor.

What then is the nuclear function for Hxk2? Our preliminary data show that several Hxk2 mutants bypass the glucose-restricted nuclear access for this enzyme. Many of these mutants are thought to be unstable and some even form nuclear aggregates. Our studies using biochemical approaches and molecular dynamics simulations suggest that the structure of Hxk2 is more flexible in the absence of glucose and Hxk2 is less stable. These are the same conditions that allow Hxk2 to access the nucleus. Based on our findings, we propose a model where in cells experiencing glucose deprivation, Hxk2 is less likely to be bound to glucose and becomes misfolded more easily. Misfolded Hxk2 accumulates in the nucleus, where the nuclear protein quality control pathways (NPQC) help degrade it. Since all glycolytic enzymes enter the nucleus, often under stress or starvation conditions, it is tempting to speculate that NPQC could more broadly regulate glycolytic enzymes.

341 **Building synthetic chromosomes from natural DNA** Alessandro L. V. Coradini, Christopher Ne Ville, Zachary A. Krieger,

Joshua Roemer, Cara Hull, Shawn Yang, Daniel T. Lusk, Ian M. Ehrenreich Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California

De novo chromosome synthesis is costly and time-consuming, limiting its use in research and biotechnology. Building synthetic chromosomes from natural components is an unexplored alternative with many potential applications. In this paper, we report CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a method for constructing synthetic chromosomes from natural components in yeast. CReATiNG entails cloning segments of natural chromosomes and then programably assembling them into synthetic chromosomes that can replace the native chromosomes in cells. We used CReATiNG to synthetically recombine chromosomes between strains and species, to modify chromosome structure, and to delete many linked, non-adjacent regions totaling 39% of a chromosome. The multiplex deletion experiment revealed that CReATiNG also enables recovery from flaws in synthetic chromosome design via recombination between a synthetic chromosome and its native counterpart. CReATiNG facilitates the application of chromosome synthesis to diverse biological problems.

342 **Simple-to-use CRISPR-SpCas9/SaCas9/AsCas12 vector series with multiple selection markers enabling single-step multiplex genome editing in budding yeast** Satoshi Okada, Emiko Kusumoto, Takashi Ito Department of Biochemistry, Kyushu University Graduate School of Medical Sciences

The CRISPR-Cas systems have become a widely used tool for genome editing across various organisms and are gaining popularity in the genetically tractable budding yeast *Saccharomyces cerevisiae*. The specificity of each CRISPR-Cas system is defined by the protospacer adjacent motif (PAM) it recognizes. This specificity can limit a system's ability to target specific regions if the corresponding PAM is not present, often requiring the use of an alternative system with a different PAM compatibility. To overcome this, distinct CRISPR-Cas systems have been effectively utilized for yeast genome editing, potentially expanding the range of editable sequences when used in combination. However, currently available plasmids were each developed according to varying design principles, which creates obstacles to their integrated application in genome editing procedures.

Here we report a plasmid vector series named LOBSTERS (League Of Backbone plasmid vector Series To Expand the Range of Selection markers for genome editing in budding yeast) constructed under a unified design principle. LOBSTERS includes a total of 21 plasmids, combining three different CRISPR-Cas systems with distinct PAMs (SpCas9, SaCas9, and AsCas12a) with seven selection markers (*HIS3*, *TRP1*, *LEU2*, *URA3*, *KanMX*, *HphMX*, and *NatMX*). All vectors are designed for Golden Gate Assembly compatibility, easing the construction of plasmids tailored to specific target sequences. Alongside these vectors, we developed a simple software tool to facilitate the design of the oligonucleotides for the Golden Gate Assembly.

These plasmids have demonstrated high efficiency in performing various editing tasks at single loci: inserting gene fragments into essential genes (median efficiency ~52%), deleting entire open reading frames (median ~79%), and introducing single amino acid substitutions (median ~79%).

The concurrent use of multiple markers enables multiplex genome editing in a single step. In dual genome editing, introducing a fluorescent protein gene at sites other than the 3' ends of essential genes resulted in a ~65% efficiency for obtaining double genome-edited clones. For triple genome editing, which involves a single-nucleotide insertion in a promoter sequence and amino acid substitution mutations in protein-coding regions, we achieved ~33% efficiency for triple genome-edited clones.

This integrated suite of vectors and accompanying software tool constitutes a comprehensive toolkit, streamlining the application of SpCas9, SaCas9, and AsCas12a for diverse genome editing tasks, particularly for those challenging to achieve through traditional yeast genetic methods.

343 **The genotype-phenotype-phenotype-phenotype map** Kerry Geiler-Samerotte Arizona State University

Predicting the phenotypic impacts of a mutation is a major goal in biology and medicine. But the paths linking genotype to phenotype are difficult to navigate. For one, some phenotypes impact others, so the impacts of mutation can stretch out across networks of related traits, percolating from impacts at the molecular level to higher orders of biological organization. Moreover, the impacts of mutation, and the networks of traits through which they spread, change across contexts like the environment or genetic background. Thus, comprehensively mapping genotype to phenotype is akin to untangling a ball of knotted strings, only to realize the task needs to be repeated many times. My lab implements and develops high-throughput phenotyping methods to simultaneously quantify the phenotypic impacts of many mutations across many contexts. In some projects, we quantify the correlations between phenotypes to infer the network through which a mutation's influence travels and how that network changes across contexts. In other projects, we deconvolute these high-dimensional data into an abstract

map that uses shared patterns of mutant behavior across contexts to make phenotypic predictions. Some of our work is driven by mechanistic hypotheses about how basic properties of cells change the phenotypic impacts of mutation in predictable ways, while other work focuses on large collections of adaptive mutations generated by laboratory evolution experiments. Our overall goal is to build predictive maps from genotype to phenotype and, in so doing, to generate insights about the map's structure (e.g., is it modular or interconnected?) and tools to study this map that will be broadly useful to the biology community.

344 ***Trans*-eQTL hotspots shape complex traits by modulating cellular states** Kaushik Renganaath, Frank W Albert Genetics, Cell Biology, & Development, University of Minnesota

Regulatory genetic variation shapes gene expression, providing an important mechanism connecting DNA variation and complex traits. However, the specific causal relationships between the expression of individual genes and complex traits remain poorly understood.

Here, we conducted a series of integrative analyses of published data from genome-wide transcriptomes and 46 genetically complex growth traits collected in the same 1,000 recombinant progeny of a cross between two strains of the yeast *Saccharomyces cerevisiae*. Loci mapped in these data account for majorities of heritability in each dataset, providing an ideal model for the comprehensive dissection of how natural genetic variation connects gene expression and growth.

We discovered thousands of genetic correlations between gene expression and growth, suggesting functional connections. Surprisingly, although local regulatory variation tends to have large effects on gene expression, it was a minor source of genetic correlations, accounting for an average of only 1% in the magnitude of these correlations.

Instead, genetic correlations tended to arise from multiple independent *trans*-acting regulatory loci across the genome. These loci accounted for 48% of the genetic correlations. In particular, *trans*-acting hotspots, which affect the expression of hundreds of genes, accounted for an average of 77% of genetic growth variation and 68% of the genetic correlations between gene expression and growth, significantly more than expected by chance.

Genes with genetic correlations were enriched for similar biological processes across traits, but the direction of effect of these enrichments differed between traits. For example, the expression of genes involved in the environmental stress response was negatively correlated with growth in a set of conditions including various carbon sources, but positively correlated with growth in conditions including environmental stressors such as high temperature or oxidative stress. Together, our results reveal a prominent role for *trans*-acting regulatory hotspots in shaping complex traits by altering central cellular states.

345 **Epigenetic context predicts gene expression variation and reproductive traits across genetically identical individuals** Amy Webster[1], John Willis[1], Erik Johnson[1], Peter Sarkies[2], Patrick Phillips[1] [1]University of Oregon, [2]University of Oxford

In recent decades, genome-wide association studies (GWAS) have been the major approach to understand the biological basis of individual differences in traits and diseases. However, GWAS approaches often have limited predictive power to explain individual differences, particularly for complex traits and diseases in which environmental factors play a substantial role in their etiology. Indeed, individual differences persist even in genetically identical individuals, although fully separating genetic and environmental causation is difficult or impossible in most organisms. To understand the basis of individual differences in the absence of genetic differences, we measured two quantitative reproductive traits in 180 genetically identical young adult *Caenorhabditis elegans* roundworms in a shared environment and performed single-individual transcriptomics on each worm. We identified hundreds of genes for which expression variation was strongly associated with reproductive traits, some of which depended on prior environmental experience and some of which was random. Multiple small sets of genes together were highly predictive of reproductive traits across individuals, explaining on average over half and over a quarter of variation in the two traits. We manipulated mRNA levels of predictive genes using RNA interference to identify a set of causal genes, demonstrating the utility of this approach for both prediction and understanding underlying biology. Finally, we found that the chromatin environment of predictive genes was enriched for H3K27 trimethylation, suggesting that individual gene expression differences underlying critical traits may be driven in part by chromatin structure. Together, this work shows that individual differences in gene expression that arise independently of underlying genetic differences are both predictive and causal in shaping reproductive traits at levels that equal or exceed genetic variation.

346 **What's so special about height? More hits ≠ more polygenic** Yuval Simons[1], Huisheng Zhu[2], Hakhamanesh Mostafavi[2,3], Guy Sella[4], Jonathan K Pritchard[2] [1]University of Chicago, [2]Stanford University, [3]New York University, [4]Columbia University

Over the past two decades, as genome-wide association studies (GWAS) systematically uncover the genetic basis of nearly every measurable human trait, height stands out as an outlier. While many traits have been associated with multiple genome-wide significant genetic variants (GWAS hits), height has the most hits (over 10,000) by quite a long way. ***Why?***

Using a simple model, we show that it is not the most polygenic traits that are expected to have the most GWAS hits. Instead, when a trait is highly polygenic and has many underlying variants, the effect of each variant is small. For the most polygenic traits, effects are so small that very few variants are discovered as GWAS hits. In contrast, for traits with few underlying common variants most are discovered as GWAS hits, but there are few common variants to discover. Therefore, it is "goldilocks" traits, with many but not too many underlying common variants, that have the most hits. We show that human height is the most heritable of human "goldilocks" traits, explaining why it has the most hits.

347 **Causal interpretations of family GWAS in the presence of heterogeneous effects** Carl Veller[1], Molly Przeworski[2,3], Graham Coop[4,5] [1]Department of Ecology & Evolution, University of Chicago, [2]Department of Biological Sciences, Columbia University, [3]Department of Systems Biology, Columbia University, [4]Department of Evolution and Ecology, University of California, Davis, [5]Center for Population Biology, University of California, Davis

Family studies have emerged as a gold standard for assessing causal effects of alleles and polygenic scores in genome-wide association studies (GWAS). Notably, an analogy is often made between the random transmission of alleles to children in families and a randomized controlled trial, on the basis of which family studies are claimed to provide an unbiased estimate of the average causal effect (or "average treatment effect"; ATE) of an allele. Here, we show that this interpretation does not hold in general. Because Mendelian segregation only randomizes alleles among children of heterozygotes, the effects of alleles in the children of homozygotes are not observable. Consequently, if an allele has different average effects in the children of homozygotes and heterozygotes, as can arise in the presence of gene by environment interactions, gene by gene interactions, or differences in LD patterns, family studies provide a biased estimate of the average effect in the population. At a single locus, family-based association studies can be thought of as providing an unbiased estimate of the average effect in the children of heterozygotes (i.e., a "local average treatment effect"; LATE). However, this interpretation does not extend to polygenic scores, where different sets of SNPs are heterozygous in each family; therefore, other than under very specific conditions, the within-family regression slope of a PGS cannot be assumed to provide an unbiased estimate for any subset or weighted average of families. Instead, family-based studies can be reinterpreted as enabling an unbiased estimate of the extent to which Mendelian segregation at loci in the PGS contributes to the population-level variance in the trait. Such statements are on a solid causal footing, but apply only to about half of the variance that could be explained by the PGS. In practice, the potential biases of family studies are likely smaller than those arising from confounding in standard, population-based GWASs, and so this approach remains important for understanding genetic contributions to phenotypic variation. Nonetheless, the causal interpretation of family-based GWAS estimates is less straightforward than has been widely appreciated.

348 **Using the threshold trait model of quantitative genetics to understand the evolutionary dynamics of dispersal in wing-dimorphic insects** Lisa Treidel[1], Caroline M Williams[2], Colin J Meiklejohn[1], Kristi M Montooth[1] [1]School of Biological Sciences, University of Nebraska, Lincoln, [2]Integrative Biology, University of California, Berkeley

Dispersal influences broadscale biogeographical, ecological, and evolutionary patterns and processes. Yet, because dispersal strategies involve complex morphological, physiological, and behavioral traits, our understanding of their evolutionary causes and consequences remains incomplete. Wing-dimorphic insect species in which discrete dispersing long-winged (LW) and non-dispersing short-winged (SW) morphs co-occur within populations provide a potential model to elucidate the evolution of alternative dispersal strategies. Using a recently resolved phylogeny, we conducted an ancestral trait reconstruction, of wing dimorphism in North American Field crickets (*Gryllus* spp.), a monophyletic clade of 45 genetically distinct lineages that diverged from a common ancestor only 1-2 million years ago. This analysis revealed a highly dynamic evolution with repeated evolutionary gains and losses of wing dimorphism across this species radiation. We further investigated the quantitative genetic basis of morph determination in the wing-dimorphic variable field cricket, *Gryllus lineaticeps*, using a family design with crosses of all possible morph combinations (LWxLW; SWxSW; LWxSW; SWxLW) and quantifying offspring morph frequencies and adult flight muscle maintenance. As predicted for a heritable trait, the frequency of LW offspring was significantly less in SWxSW relative to LWxLW families. The frequency of LW offspring and the maintenance of adult flight muscles was also positively correlated across families, indicating a common genetic basis of dispersal-related traits. Morph frequencies among females were more LW-biased compared to males, but males were more likely to maintain their flight muscles in early adulthood than were females. We interpret the results of both analyses using a threshold trait framework and hypothesize that these evolutionary patterns arise because morph determination is a threshold trait, controlled by an underlying unobservable environmentally sensitive quantitative trait, called liability, and a threshold. For wing dimorphisms, when liability exceeds a threshold during a critical period in development, wing and flight muscle development are proposed to be inhibited resulting in the flightless short-wing morph. The control of multiple flight related traits by liability could explain

their coordinated evolution and shifts in morph frequencies between sexes or species can evolve quickly via changes in liabilities or thresholds.

349 **Clark (2023) and the Persistence of Hereditarian Fallacies** Jedidiah Carlson[1,2], John W Benning[3], Ruth G. Shaw[4], Arbel Harpak[1,2] [1]Department of Integrative Biology, University of Texas at Austin, [2]Department of Population Health, University of Texas at Austin, [3]Department of Botany, University of Wyoming, [4]Department of Ecology, Evolution, and Behavior, University of Minnesota

Understanding of the persistence of social status in families is contentious. Scholarly assertion that such persistence is rooted in genetic variation was foundational to the development and implementation of eugenic policies in the 20th century. Hereditarian claims of an outsized role for genetics in social outcomes have continued to maintain a foothold among academics. In a recent paper, Clark (2023) measures correlations between relatives in a variety of socioeconomic status indicators, drawing on data from >400,000 individuals with rare surnames from census and population registers spanning four centuries in England. Based on a fit of these familial status correlations to a classic quantitative genetics model (Fisher, 1918), the paper argues that: (1) variation in socioeconomic status is largely determined by additive genetic variation; (2) contemporary English people "remain correlated in outcomes with their lineage relatives in exactly the same way as in preindustrial England"; and (3) social mobility has remained static over this time period due to strong assortative mating on a "social genotype."

We reanalyze the data of Clark (2023) and show that these conclusions are based on the flawed assumption that genetic inheritance is the *only* transmissible source of familial resemblance in social status. We demonstrate that these correlation estimates conflate genetic and non-genetic transmission (e.g., of wealth) within families, and how this confounding leads to the misconstrual of model parameters and fundamental errors of inference. Inconsistent with claims (2) and (3), we show that familial correlations in status are variable—generally decreasing—through the time period analyzed. Finally, we discover statistical artifacts in the data that substantially bias these correlation estimates. All these considerations, but particularly the first, invalidate use of, such data for disentangling the roles of genetic and non-genetic factors. We conclude that Clark (2023) tells us nothing about the genetic and non-genetic transmission of social status, and discuss the impacts of these and other hereditarian fallacies resurfacing in recent literature.

350 **Revealing functional consequences of eukaryotic histone H2A repertoire innovations** Pravrutha Raman[1], Sierra Simmerman[2], Hana Khan[3], Kelsey Woodruff[4], Amy Hamada[3], Toshio Tsukiyama[5], Harmit S Malik[2] [1]Basic Sciences, Fred Hutchinson Cancer Center, [2]Fred Hutchinson Cancer Center and HHMI, [3]University of Washington, [4]University of Washington, Fred Hutchinson Cancer Center, [5]Fred Hutchinson Cancer Center

Given their essential roles in fundamental biological processes, histones would be predicted to be highly conserved. Yet, histone repertoires can vastly differ across lineages due to gene duplication, gene loss, and sequence divergence. The genetic innovation of histone repertoires could confer lineage-specific adaptive advantages. Here, we investigate the unusual case of a fusion of two well-conserved histone H2A variants. Most eukaryotes possess an H2A repertoire of canonical H2A and two distinct variants H2A.Z and H2A.X, which participate in gene regulation and DNA damage response (DDR), respectively. In contrast, all *Drosophila* species encode a single H2Av gene, a functional chimera of H2A.X and H2A.Z. Our evolutionary analyses show that H2Av arose in a subset of *Diptera* (winged-insects) by *H2A.Z* acquiring the *H2A.X* C-terminal tail containing "SQ motif" required for DDR. Thus, DDR and gene regulation functions of H2A.X and H2A.Z are now confined to the same genomic location in some *Diptera*. We also find that this fusion has duplicated and acquired distinct functions in some *Drosophila* species. We hypothesize that this unusual fusion may provide an adaptive advantage by enhancing DNA repair in H2A.Z-enriched genomic regions, restricting transposition that rely on DDR to gene-poor heterochromatin in *Drosophila* genomes. To investigate this possibility, we are reversing the *D. melanogaster* H2A repertoire to its ancestral *Diptera* state by engineering distinct H2A.X and H2A.Z. This uncouples their functions and will reveal how genomic functions are altered by their fusion. We also recreated this unique evolutionary event in yeast by appending the SQ motif from yeast H2A.X onto its H2A.Z. These 'fly-like' yeast have growth rates indistinguishable from wild-type. However, a fly-like H2A repertoire dramatically reduces DDR efficiency that cannot be attributed entirely to a lack of SQ phosphorylation, since phosphomimic EQ mutations fail to enhance DDR. Thus, an H2A+H2Av repertoire impairs DDR in yeast. We are analyzing how transposition and DDR-dependent processes like meiosis are altered in fly-like yeast. Our yeast data raise the possibility that an H2A+H2Av repertoire might lead to a trade-off of compromised DDR in fly genomes and to the co-evolution of other fly chromatin factors to facilitate DDR. Together, our work reveals the unexpected evolution of conserved, essential H2A variants and their functional consequences on genome regulation and instability.

351 **The Mute button: Turning down the volume of histone expression** Mark S Geisler[1], William F Marzluff[2], Robert J Duronio[3] [1]Curriculum in Genetics and Molecular Biology, University of North Carolina - Chapel Hill, [2]Department of

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Replication of a eukaryotic genome requires the production of hundreds of millions of histone proteins to package the newly synthesized DNA. To meet this large demand for histone production, all eukaryotes coordinate high level expression of replication-dependent (RD) histone gene clusters during S-phase of the cell division cycle. Regulating expression levels of RD-histone genes during S-phase is important for maintaining genomic integrity and normal cell cycle progression. This regulation, along with expression and processing of the histone RNA, occurs within a biomolecular condensate that assembles at RD-histone genes called the Histone Locus Body (HLB). Expression of RD-histone genes is activated during S phase by CyclinE/CDK2 phosphorylation of the HLB scaffold protein, MXC. However, much less is known about how histone transcription is terminated as cells exit S-phase. The HLB component Mute is a negative regulator of histone gene expression. Mutation of *mute* causes embryonic lethality accompanied by an increase in the steady state amount of RD-histone mRNA. By combining EdU labeling with fluorescent in situ hybridization (FISH) to core histone mRNAs we found that loss of Mute leads to inappropriate expression of RD-histone genes during G2 phase of the cell cycle in the embryonic ventral nerve cord. Interestingly, these G2 cells retain MXC phosphorylation at the HLB, suggesting that Mute terminates RD-histone transcription at the end of S-phase by removing or blocking the CyclinE/CDK2 dependent phosphorylation of MXC. By generating genetically mosaic larval imaginal discs, we found Mute null cells have a severe proliferation defect, showing that limiting RD-histone expression to S-phase is critical for normal cell cycle progression and proliferation. As in the embryo, these *mute* null cells also display an uncoupling of histone gene transcription from S-phase. These data lead us to hypothesize that Mute restricts RD-histone expression to S-phase by removing or blocking CyclinE/CDK2 phosphorylation of MXC to repress histone gene expression as cells transition into G2. We are currently exploring whether misexpression of histone genes affects genome organization throughout embryogenesis using ATAC-Seq. This work enhances our understanding of the role of RD histone gene regulation in metazoans and provides insight into the mechanisms that govern the regulation of this highly conserved cellular process.

352 **Ectopic transcription due to inappropriately inherited histone methylation may interfere with the ongoing function of terminally differentiated cells** Monica Reeves, Juan Rodriguez, Eilleen Falkenberry, David Katz Emory University

C. elegans larvae that are double mutant for the H3K4 demethylase *spr-5* (LSD1/KDM1A in mammals) and the H3K9 methyltransferase *met-2* (SETDB1 in mammals) exhibit L2 developmental delay, chemotaxis defects, muscle defects and a failure to elongate the gonad. We found that these *spr-5;met-2* phenotypes are caused by the ectopic expression of germline genes in somatic tissues, which begins during embryonic stages. This provided the unique opportunity to determine how ectopic expression, due to inappropriate histone methylation, interferes with the invariant *C. elegans* embryonic lineage. Surprisingly, using automated lineage tracing, we find that *spr-5;met-2* mutants have no embryonic lineage defects through the 200-cell stage, despite the ectopic germline expression and the major phenotypes we observe one cell division later, in larvae. This suggested that the *spr-5;met-2* phenotypes are not due to development defects. Instead, they may be due to ongoing functional defects in terminally differentiated cells. To test this directly, we used RNAi to shut off the ectopic expression of germline genes in L2 *spr-5;met-2* larvae, which have a fully formed nervous system. Remarkably, we find that shutting off the ectopic germline expression rescues normal chemotaxis behavior in the same adult worms that previously had a chemotaxis defect at the L2 stage. This suggests that ongoing ectopic transcription due to the misregulation of epigenetic inheritance can block normal behavior in a fully intact nervous system. Interestingly, some of the *spr-5;met-2* phenotypes, such as the developmental delay and behavior abnormalities, broadly overlap with those observed in human Kabuki Syndrome-like patients, caused by mutations in *Lsd1* and other histone modifying enzymes. Our data in *C. elegans* suggest that it may be possible to rescue some of the defects in human patients by reverting the chromatin in adults. Similar to what we observe in *C. elegans*, we found that partially compromising LSD1 maternally in mice also results in inherited phenotypes that manifest postnatally, including perinatal lethality, developmental delay, craniofacial defects and altered behavior. We are currently using this new mouse paradigm to investigate whether some of the observed phenotypes are due to ongoing epigenetic defects in differentiated tissues.

353 **OVO Positively Regulates Essential Maternal Pathways by Binding Near the Transcriptional Start Sites in the *Drosophila* Female Germline** Leif Benner, Savannah Muron, Jillian G Gomez, Brian Oliver National Institutes of Health

Differentiation of female germline stem cells into a mature oocyte includes the expression of a number of RNAs and proteins that drive early embryonic development in *Drosophila*. We have little insight into what activates the expression of these maternal factors. One candidate is the zinc-finger protein OVO. OVO is required for female germline viability and has been shown to positively regulate its own expression, as well as a downstream target, *ovarian tumor*, by binding to the transcriptional start site (TSS). To find additional OVO targets in the female germline and further elucidate OVO's role in oocyte development, we performed ChIP-seq to determine genome-wide OVO occupancy, as well as RNA-seq comparing

hypomorphic and wild type rescue *ovo* alleles. OVO preferentially binds in close proximity to target TSSs genome-wide, is associated with open chromatin, transcriptionally active histone marks, and OVO-dependent expression. Motif enrichment analysis on OVO ChIP peaks identified a 5`-TAACNGT-3` OVO DNA binding motif spatially enriched near TSSs. However, the OVO DNA binding motif does not exhibit precise motif spacing relative to the TSS characteristic of RNA Polymerase II complex binding core promoter elements. Integrated genomics analysis showed that 525 genes that are bound and increase in expression downstream of OVO are known to be essential maternally expressed genes. These include genes involved in anterior/posterior/germ plasm specification (*bcd, exu, swa, osk, nos, pgc, gcl*), egg activation (*png, plu, gnu, wisp, C(3)g, mtrm*), translational regulation (*cup*, *orb*, *bru1, me31B*), and vitelline membrane formation (*fs(1)N*, *fs(1)M3*, *clos*). This suggests that OVO is a master transcriptional regulator of oocyte development and is responsible for the expression of structural components of the egg as well as maternally provided RNAs that are required for early embryonic development.

354 **Sexually dimorphic Argonaute structure and localization facilitate sex specificity of small RNA pathways in *C. elegans* germ cells** Acadia L DiNardo[1], Hannah Wilson[2], Rachael Giersch[2], Nicole A Kurhanewicz[2], Diana E Libuda[2] [1]Biology, University of Oregon, [2]University of Oregon

Germ cell proliferation and proper genome inheritance are critical to maintain fertility through generations. To promote proper germ cell development and fertility, small RNA pathways employ Argonaute proteins (WAGOs) to downregulate aberrant transcripts. WAGOs are largely housed within the germ granule, a cytoplasmic, phase separated compartment adjacent to nuclear pore complexes of germ cells. While previous work found that WAGO-1 post-transcriptionally regulates distinct sex-specific gene targets, the mechanism by which WAGO-1 achieves sexually dimorphic gene regulation is largely unknown. Here we show that WAGO-1 both responds to and regulates sexually dimorphic germ granule structure and function. During meiotic prophase I progression, we find that the structural germ granule components (PGL-1, PGL-3, and ZNFX-1) and the 21U small RNA regulator PRG-1 display dynamic and distinct localization patterns between egg and sperm development that elicit differential WAGO-1 localization and structure. Only during spermatogenesis, PGL-1, ZNFX-1, and WAGO-1 are encompassed within a shell of PRG-1 and a ring of PGL-3. These striking protein organizations encircling the germ granule suggest sex-specific roles of PGL-3 and PRG-1 in regulating germ granule structure. Moreover, we demonstrate that WAGO-1 is able to dimerize only during spermatogenesis when it resides within the PGL-3 and PRG-1 rings, which may facilitate the spermatogenesis-specific dimerization of WAGO-1. Further, we find that the N- and C-terminus of WAGO-1 play sex-specific roles in regulating the stability of germ granules. Additionally, our results indicate that disruption of the intrinsically disordered region of the N-terminus of WAGO-1 causes PGL-1 to lose phase separation only during spermatogenesis. Moreover, deletions of the PIWI-domain containing C-terminus causes complete sterility only in males. Together, our studies suggest that sexual dimorphisms in the structure and stability of the germ granule, where small RNAs are processed, may affect end-terminal interactions of Argonautes to enable sex-specific gene expression profiles required for fertility.

355 **A regulatory network of Sox and Six transcription factors initiate hearing regeneration in zebrafish** Sarah Hanson[1], Jeffrey Baffoe-Bonnie[2], Michelle Macurak[1], Braveen Joseph[2], Shawn Burgess[2], Erin A Jimenez[1] [1]Biology, Johns Hopkins University, [2]National Human Genome Research Institute, National Institutes of Health

Millions of people world-wide experience hearing or balance disorders due to the loss of hair cells in the inner ear. The hair cells are the mechanosensory cells used in the auditory and vestibular organs of all vertebrates and lateral line systems of aquatic vertebrates. In zebrafish and other non-mammalian species, hair cells turn over during homeostasis and regenerate completely after being destroyed or damaged by acoustic or chemical exposure, while in mammals, destroying or damaging hair cells results in permanent hearing loss or vestibular dysfunction.

To understand hearing regeneration, it is essential to understand how genes respond to injury and how those responses are controlled in the genome. To study this phenomenon closely, we previously performed a targeted ablation of the mechanosensory receptors in adult zebrafish auditory and vestibular organs and characterized the epigenome and transcriptome at consecutive time-points during regeneration. We identified unique, cell-specific transcription factor (TF) motif patterns in the chromatin that opened specifically during regeneration. We correlated this emergent enhancer activity with differential gene expression to identify key gene regulatory networks driving regeneration. We detected a clear pattern of overlapping Sox- and Six- family TF gene expression and binding motifs, suggesting a combinatorial program of TFs driving regeneration and cell identity.

Despite significant progress in identifying key genes involved in hair cell regeneration, there is still a gap in our knowledge regarding the role of the gene regulatory networks involved in this process. In this study, we investigated if Sox- and Six-TFs and identified regeneration-responsive regulatory regions containing Sox- and/or Six- binding motifs, were necessary in zebrafish hair cell regeneration by testing function through gene knockouts and mass mutagenizing putative enhancer regulatory regions. Using CRISPR/Cas9 guided mutagenesis, we phenotypically characterized TF mutants, singly and in

combinations, for defects or improvements in lateral line development and hair cell regeneration. To accelerate screening for hair cell regeneration phenotypes associated with enhancers containing either Sox, Six, or both motifs, we employed MIC-Drop (Multiplexed Intermixed CRISPR Droplets) to mass mutagenize putative regeneration-responsive regulatory regions and screened for hair cell regeneration defects using the lateral line as a proxy for large scale testing. Finally, we used fluorescent *in situ* hybridization to gain spatial resolution and determine the expression pattern of *sox* and *six* genes in the inner ear and lateral line. By combining knockout strategies, phenotypic analysis, and TF gene expression, we are beginning to understand the combinatorial "code" of TFs that initiate regeneration and instruct hair cell differentiation.

356 **Ketogenic diet and multiple loci drive diet-induced thermogenesis in mice** Alexandra Naron[1], Anna Salvador[2], David Threadgill[1] [1]Cell Biology and Genetics, Texas A&M University, [2]University of North Carolina-Chapel Hill

Precision nutrition is the customization of nutrition guidance through individualized plans based on unique metabolic characteristics to improve general health. However, there is still a significant lapse in knowledge of how genetics impacts diet. Genetically heterogenous mouse models have demonstrated significant variation in metabolism, activity, and body fat composition across different strains and diets. Genetic regulation of thermogenesis may contribute to the variation seen in response to diet. Investigating the role that genetics plays in individual consumption of energy, production of heat, and body composition will provide knowledge to better individualize nutritional approaches.

Previously C57BL/6J (B6), A/J, FVB/NJ (FVB), and NOD/ShiLtJ (NOD) mice were exposed to human comparable diets varied in macronutrient content, including an American (high fat, high carbohydrate) and ketogenic (high fat, no carbohydrate) diet to investigate the impact diet plays on thermogenesis rates.

We observed that the ketogenic diet increased rates of heat expenditure in A/J mice without a corresponding increase in activity relative to their A/J counterparts exposed to the American diet. B6 mice showed a more modest response to the ketogenic diet relative to the American diet with regards to thermogenesis rates. Percentage of body fat was also observed to have decreased in both A/J and B6 mice fed the ketogenic diet relative to their counterparts exposed to the American diet.

To further investigate genetic regulation of diet-induced thermogenesis, an F2 population of C57BL6/J x A/J was generated. F2s were genotyped at 7854 markers on Mouse Universal Genotyping Array (MUGA). Unconditioned linkage analysis was conducted with R/qtl2 and revealed several overlapping quantitative trait loci (QTL). QTL overlapping on Chr 7 were identified for heat expenditure and body fat gain. QTL overlapping on Chr 1 were identified for activity and heat expenditure. Allele effects showed AJ/AJ alleles driving higher heat expenditure at *Heatq2*, B6/B6 alleles driving higher activity at *Actq1*, and B6/B6 alleles driving higher percentage of body fat gain at *Bfgq3*. Investigation of an interaction between *Heatq2* and *Actq1* showed that F2 mice that are AJ/AJ at *Heatq2* and B6/B6 at *Actq1* show higher heat expenditure and lowest body fat gain.

To narrow the overlapping QTL of interest, we are generating a subconsomic line from chromosome substitution strains (CSS) for A/J Chr1 and Chr7. This will allow us to validate previously identified QTLs of interest and assist in the identification of genes regulating diet-induced thermogenesis.

357 **Nucleoporin complex NPP-14/24 shape perinuclear germ granule architecture and coordinate piRNA silencing** Kun Shi[1], Zhenzhen Du[1], Xinyu Fan[1], Jordan Brown[2], Ying Zhang[1], Heng-Chi Lee[2], Donglei Zhang[1,2] [1]Biochemistry and Molecular Biology, Huazhong University of Science and Technology, [2]Molecular Genetics and Cell Biology, University of Chicago

Germ granules are RNA-rich biomolecular condensates containing factors for the posttranscriptional regulation of gene expression and the development of germ cells [1]. In *C. elegans*, germ granules are associated with nuclear pore clusters [2]. Previous studies have shown that assembly of P granules in the perinuclear region is critical for the fidelity of piRNA-mediated gene silencing [3]. However, the mechanism by which germ granules associate with the nucleus and the functional relevance of such an association remain unknown. Here we showed that the conserved NPP-14/24 nucleoporin complex, which is localized on the cytoplasmic side of the nuclear pore complex, recruited various perinuclear germ granules to nucleus. Depletion of NPP-14/24 led to disaggregation of nuclear pore clusters and dissociation of most perinuclear germ granules. Additionally, germ granule components that originally localized in distinct P/Z/S/M foci merged into few remaining large condensates at the perinuclear region. Our results showed that poly(UG)-tailed RNAs, the RNA degradation intermediates, accumulated in the enlarged perinuclear condensates, suggesting that these granules are capable of RNA silencing. Interestingly, using a piRNA reporter, we found that piRNA-mediated gene silencing was enhanced in *npp-14/24* mutants. Notably, WAGO 22G-RNAs were specifically elevated at piRNA target sites across transcriptome, suggesting enhanced production of piRNA-dependent WAGO 22G-RNAs. On the other hand, we observed that *npp-14/24* mutants exhibited defects in siRNA-triggered transgenerational gene silencing, consistent with the hypothesis that the piRNA pathway antagonizes the inheritance of siRNA-mediated gene silencing [4]. Using proximity labeling, we further identified an uncharacterized P granule factor with intrinsic disordered

domains, GGO-1 (Germ Granule Organizer-1), associating with both NPP-14 and the P granule protein PRG-1. Remarkably, *ggo-1* mutants exhibited similar phenotypes as *npp-14/24* mutants, including merged perinuclear granules and enhanced piRNA silencing with elevated WAGO 22G-RNAs near piRNA target sites. Together, our results uncover the function of the NPP-14/24 complex in spatially organizing perinuclear germ granules and shed light on how organized germ granules function in fine-tuning the piRNA-based transcriptome surveillance and the inheritance of gene silencing.

[1] Voronina et al. 2011; [2] Updike et al. 2011; [3] Chen et al. 2022; [4] Shukla et al. 2021

358 **Germ granules modify the posterior pole promoting primordial germ cell formation**

Marcus D Kilwein[1], Elizabeth Gavis[2], Stanislav Shvartsman[1] [1]Molecular Biology, Princeton University & Simons Foundation, [2]Molecular Biology, Princeton University

Specification and segregation of the germline is one of the first events in metazoan embryogenesis. In Drosophila, the primordial germ cells (PGCs) exit syncytial development an hour before the soma, by precociously cellularizing at the posterior pole. This event depends on maternally deposited germ granules (GGs). We have known since the 1970s that transplantation of the germ granules is sufficient to induce PGC formation at new regions of the embryo. However, how germ granules promote PGC formation has remained unanswered. Our data show that GGs initiate PGC formation through local plasma membrane modifications that drive f-actin dynamics and precocious cell formation.

Among mRNAs found in germ granules or otherwise enriched at the posterior are mRNAs coding for actin regulatory and interacting proteins that could be involved in PGC formation. By live imaging using the f-actin sensors GFP-MoeABD and Lifeact, I found that f-actin becomes enriched at the posterior cortex of the embryo before nuclear cycle 5. This f-actin enrichment is dependent on GGs, as embryos lacking GGs through removal of the GG organizer Oskar, do not enrich f-actin.

Furthermore, GG transplantation induces f-actin formation at the new location in the recipient embryo and leads to formation of ectopic PGCs. I next sought to determine what actin polymerization factors organize the posterior enrichment, focusing on regulators of the ARP2/3 complex which is known to generate pushing force like that seen during PGC budding. The ARP2/3 activator WASp was indeed enriched at the posterior and localized to the bud-front during PGC formation. Injection of an inhibitor for ARP2/3 showed that ARP2/3 is essential both for the posterior f-actin enrichment and formation of the PGCs.

I then sought to determine how germ granules promote f-actin enrichment. Intriguingly, 7 of the 8 actin-related mRNAs found in germ granules contained binding domains for the membrane lipid PI(4,5)P2(PIP2). PIP2 has a well-established role in promoting f-actin formation. Using the PIP2 binding sensor PH-mCherry, I found enrichment for PIP2 at the posterior pole. GG transplantation induced PIP2 synthesis at the new location in the recipient embryo and formation of ectopic PGCs. Further, the PIP2 producing enzyme dPIP5K was enriched at the posterior pole, in a similar manner to PH-mCherry. Finally, injection of PIP2 at a new region of the embryo induced futile f-actin furrows at a new location.

359 **Homologous recombination is essential for DNA damage-induced regeneration of germline stem cells in the Drosophila testis niche**

Jasmine Grey[1], Salman Hasan[2], Janelle Bellot[1], Erika Matunis[1] [1]Cell Biology, Johns Hopkins University School of Medicine, [2]Johns Hopkins University School of Medicine

Germline stem cells (GSCs) transmit the genome to the next generation, and therefore understanding their DNA repair strategies is of particular interest. Several highly conserved DNA repair pathways sense and repair double strand breaks (DSBs), which are otherwise lethal events. This includes homologous recombination (HR), which uses the sister chromatid as a template to yield high fidelity repair, and non-homologous end joining (NHEJ), which is often mutagenic. Whether stem cells differentially require specific DSB repair pathways is not typically studied within tissues. Here, we use the *Drosophila* testis stem cell niche to study the responses of GSCs to DNA DSBs. We first established conditions to follow GSC loss and recovery after ɣ-irradiation (IR) *in vivo*. Exposing adult male flies to 75 Gy of IR causes approximately half of GSCs to become lost from the niche within 24 hours. Lost GSCs are replenished and their progeny (spermatogonia) return by one week, indicating GSC functionality. Unexpectedly, TUNEL staining and p35 overexpression show that GSC loss is not due to apoptosis. Instead, live imaging and clonal analysis show that IR causes single GSCs to detach from the niche and move toward the base of the testis, an event not seen in unirradiated testes. To identify the DNA repair pathway(s) at play, we assayed GSC loss and recovery in several mutant backgrounds. GSCs null for Chk2 or p53 persist in the niche after IR, consistent with a requirement for these factors in DSB sensing. Since the detachment of some GSCs from the niche is likely downstream of p53 activation, we analyzed GSC recovery in flies null for either NHEJ or HR pathway members. As expected, GSCs unable to perform NHEJ are partially lost and then replenished after IR exposure, as in wild type testes. Unexpectedly, GSCs unable to perform HR are not maintained within the niche. The GSC requirement for HR is cell autonomous, aligning with the germline's unique role in maintaining genomic integrity. We are now investigating the significance of the cell cycle phase on stem cell survival after IR. A deeper understanding of stem cell resistance to IR has implications in other radio-resistant stem cells, like cancer stem cells, which contribute to the regrowth of tumors after radiotherapy.

360 **An actomyosin network organizes niche morphology and responds to feedback from recruited stem cells** Bailey N Warder[1], Kara A Nelson[1], Justin Sui[2], Lauren Anllo[3], Stephen DiNardo[1] [1]Cell and Developmental Biology, University of Pennsylvania, [2]Department of Medicine, University of Pittsburgh, [3]Department of Biology, East Carolina University

Stem cells often rely on signals from a niche, which in many tissues adopts a precise morphology. What remains elusive is how niches are formed, and how morphology impacts function. To address this, we leverage the *Drosophila* gonadal niche, combining genetic tractability with live-imaging. This niche adopts a distinct morphology during embryogenesis, with a smoothened boundary between itself and adherent germline stem cells (GSCs). The niche plays key roles in regulating GSC behavior and it is thus vital to identify mechanisms of niche formation. We have found that the niche-GSC boundary is enriched for F-actin and Myosin II (MyoII). We therefore hypothesize that actomyosin contractility (AMC) shapes the niche, and makes it more efficient in guiding GSC behavior. Through transgenic and pharmacological manipulations, we show that AMC is required for generating a smoothened niche-GSC boundary and therefore a proper niche shape. Additionally, we have evidence that proper niche shape is crucial for its functions of sending self-renewal signals to a subset of germ cells, as well as regulating GSC divisions. Since AMC is therefore vital for forming a functional niche, our work further addresses mechanisms that robustly polarize MyoII in the niche. Interestingly, MyoII polarity can be regulated by mechanical forces exerted on a cell, and we have evidence that GSC divisions are required for MyoII polarity and niche morphogenesis. We therefore suspect that proper MyoII polarity in the niche is in part induced by forces inherent to GSC divisions. Our work unveils a unique feedback mechanism where stem cells shape the niche that guides their behavior. This work was supported by NIH grants F31HD105342 and T32GM007229 to BNW; T32 HD083185 and F31 HD111208 to KAN; GM125123 to LA; R35 GM136270 to SD.

361 **Autophagy in Meiotic Fidelity and Genome Integrity** Kaitlin Kosinski[1], Marilina Raices[2], Judith Yanowitz[3], Alicia Melendez[1] [1]Biology, Queens College/City University of NY, [2]Dept of OB/GYN/Reproductive Sciences, University of Pittsburgh School of Medicine, [3]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh School of Medicine

Autophagy is a conserved cellular recycling process crucial for homeostasis. In this multistep process, cellular material destined for degradation is enclosed in the autophagosome, a double membrane-bound organelle that fuses with the lysosome for degradation. Reproduction is an energetically expensive process for living organisms, and the decision for an organism to invest in its offspring or survival thus requires the communication between the conditions in the environment and the germ line. Although typically upregulated in response to cellular stress conditions, basal levels of autophagy can also function as a quality control mechanism that serves to maintain cellular homeostasis in response to developmental and environmental cues. The role of autophagy in cellular homeostasis is well documented, but its function in the maintenance of genomic stability in the germline remains unclear. We recently described roles for BEC-1, the *C. elegans* ortholog of mammalian BEC-1/BECN1, and several other autophagy genes in germline stem cell homeostasis (Ames et al., 2017). Specifically, we found that BEC-1, as well as ATG-18 (in mammals, WIPI1/2), ATG-16.2 (ATG16L) and ATG-7 (ATG7), are required for the late larval expansion of germline stem cell progenitors during development. We have also reported a role for BEC-1 in the DNA damage response (DDR) to UV, where it exhibits crosstalk with CEP-1, the *C. elegans* ortholog of p53 (Hoffman et al., 2014). We have now found that autophagy genes are also required to maintain meiotic fidelity. In *bec-1, atg-7, unc-51* or *atg-18* mutants, the diakinesis oocytes display chromosomal abnormalities, defects in meiotic fidelity. CEP-1 is well known for its roles in promoting genomic integrity through the DDR, including cell cycle control, apoptosis, and DNA repair. CEP-1 also acts in meiosis, in the double-strand break formation and suppressing nonhomologous end-joining (NHEJ), to ensure the high-fidelity repair of double-strand breaks (DSBs) by the error-free homologous recombination (HR) pathway (Mateo et al., 2016). We are now investigating the mechanism(s) by which BEC-1 and autophagy genes promote DNA damage repair in germline development. Our current work seeks to elucidate which tissues require autophagy to regulate meiosis and germline proliferation and at which step of meiosis autophagy genes are required to regulate the DNA damage response during germline development.

362 **Epithelial migration speed modulates basement membrane mechanics to control the degree of tissue elongation** Mitch Anderson, Sally Horne-Badovinac University of Chicago

Basement membranes (BMs) are sheet-like extracellular matrices that line epithelia and other tissues to support their function. It is increasingly appreciated, however, that epithelia can also pattern the mechanical properties of their BM to control their own morphogenesis, but how this occurs is poorly understood. In this work, we use the *Drosophila* egg chamber to ask how a BM is patterned to control tissue elongation. An egg chamber is composed of a germ cell cluster surrounded by an epithelium of follicle cells; the entire organ is encased by a BM. Though initially spherical, egg chambers elongate throughout their development to produce an ellipsoid egg. This morphogenesis depends on a collective migration of the follicle cells across the BM. During this process, cell movement synergizes with new BM protein secretion to create an array of fibrils in the matrix, which stiffen the BM over the center of the egg chamber and allow it to act as a “molecular corset” to preferentially direct tissue growth along one axis. When follicle cell migration is blocked, as occurs with loss of *fat2*, the

BM corset fails to form, and the egg is much rounder than in wild type. Here, we leverage a different mutant condition, loss of *Sema5c*, to show that the mechanical properties of the BM can also be tuned to produce other morphogenetic outcomes. We previously showed that loss of *Sema5c* leads to slowed follicle cell migration and mildly round eggs. We have now found that the difference in egg shape between the two mutant conditions occurs because *Sema5c* egg chambers continue to elongate over a longer developmental time window than *fat2* egg chambers. We then explored the mechanical properties of the BM in *Sema5c* egg chambers by assaying its ability to resist rupture in response to acute osmotic swelling of the tissue. We found that *Sema5c* BMs become defective in this assay shortly before the defect in egg chamber elongation appears, and this phenotype is likely due to a reduction in BM fibrils. Restoring fibrils to the BMs of *Sema5c* egg chambers by changing the subcellular location of BM protein secretion suppresses the egg elongation defect. By contrast, reducing the levels of the primary BM component, Collagen IV, enhances the elongation defect without further disrupting cell migration. Altogether, these results suggest that the speed at which the follicle cells migrate affects BM remodeling and mechanics in a way that can be harnessed to generate a range of tissue shapes.

363 **The function of Bucky ball and the Balbiani body in oocyte polarity and germ cell development** Manami Kobayashi, Mary C Mullins University of Pennsylvania

Oocyte polarity is important for formation of the embryonic body axis and germ cells. The Balbiani body (Bb) is a large, membrane-less, mitochondrial-rich, electron-dense structure observed in the primary oocyte, which is conserved from insects to mammals, including humans. However, its function and the mechanisms regulating it are not fully understood. In frogs and fish, the Bb is postulated to establish oocyte polarity. mRNAs essential for body axis formation and germ cell formation localize to the Bb. As the Bb disassembles at the cortex in later stage oocytes, the Bb-localized transcripts and proteins become docked at the oocyte cortex. These vegetally-localized factors delivered by the Bb are postulated to specify the animal-vegetal (AV) axis. The Bb localized protein, Bucky ball (Buc), is the only gene known to function in Bb formation. Here, I established *buc* CRISPR mutant lines that are *buc* hypomorphic alleles, which differ to null alleles. In *buc* null mutants, AV polarity fails to form and the animal pole is expanded radially in the oocyte and embryo, which dies before 1 dpf. In *buc* hypomorphic mutants, embryos displayed normal AV polarity and survived to 1 dpf; however, many were severely ventralized. Those embryos with a WT phenotype failed to generate primordial germ cells and developed into sterile males. In *buc* hypomorphic oocytes, the mitochondria did not aggregate into the Bb and the Bb failed to form in early stage oocytes. Interestingly, in later stage oocytes, Buc and transcripts localized to the vegetal cortex, although in a reduced size domain. In these later stage oocytes the animally-localized mRNA, *cyclin B1*, was localized in an expanded animal pole domain. These results demonstrate the importance of Buc in germ cell formation and dorsal axis formation and suggest a Bb-independent mechanism of polarizing the oocyte.

364 **Dysfusion suppresses border cell recruitment and migration by attenuating Stat nuclear import** Anna C.-C. Jang[1], Jhen-Wei Wu[1], Chueh-Wen Wang[1], Ruo-Yu Chen[1], Liang-Yi Hung[1], Yu-Chen Tsai[2], Yu-Chiuan Chang[3] [1]Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, [2]Department of Life Science and Life Science Center, Tunghai University, [3]Institute of Biomedical Sciences, National Sun Yat-sen University

Collective cell migration, in which cells move as a group, is a pivotal process of embryonic development, wound healing and cancer dissemination. *Drosophila* Border Cells (BCs) escape from the neighboring cells, form a cluster and migrate through germ cells, serving as a good model to investigate cohesive cell movement. Jak/Stat signaling establishes follicle cell patterning in early oogenesis but induces BC migration at the later stage. The underlying mechanism governing such morphogenic switch remains to be explored. Here, we report that the spatiotemporal downregulation of *dysfusion* (*dysf*) escalates Jak/Stat activity, which thereby turns epithelial follicles into migratory BCs in egg chambers. Dysf protein is located on the inner nuclear membrane of all germline and follicle cells, but gradually reduced in BCs upon migration. When BCs reach the oocyte border, their Dysf staining becomes undetected. Overexpression of UAS-*dysf* severely impaired BC recruitment; conversely, *dysf* loss-of-function induced extra BCs. A similar phenotype of ectopic BCs caused by Jak/Stat hyperactivation can be suppressed by *dysf* overexpression. Furthermore, up-regulation of Dysf impeded the phosphorylation, transcriptional activity, and nuclear import of Stat protein, indicating that *dysf* negatively regulates Jak/Stat signaling. To further unfold Dysf-mediated nucleocytoplasmic translocation of Stat, we performed a biochemical screen to seek for proteins which interact with Dysf, and identified Pendulin (Pen), a member of the Importin-alpha protein family. GST pull-down assays revealed direct interaction of Dysf and Pen via the importin beta binding (IBB) domain. Elevating Pen expression counteracted the effect of *dysf* up-regulation to increase the flow of Stat into the nucleus. Therefore, we propose that *dysf* restricts Jak/Stat signaling activity to confine the size of BC clusters by constraining Stat nuclear transport. More importantly, we also demonstrated that the human homologue of Dysf, NPAS4, executes a similar task to prevent nuclear accumulation of STAT3 and impairs STAT3-induced migration in liver and colon cancer cell lines.

365 **Elucidating the molecular mechanisms of midbody reorganization during ring canal biogenesis** Kari L. Price, Ellery

Winkler, Kathleen Ayers, Yasmin Almazan, Lynn Cooley Yale School of Medicine

Animal gametes typically develop through some or all of gametogenesis as syncytia of cells attached by stable intercellular bridges called ring canals. Ring canals are important conduits allowing the movement of mRNAs, proteins, and organelles between sibling cells. Despite the conservation of germline ring canals across evolution, canonical cytokinesis is modified during germ cell division to produce ring canals has been poorly understood. Using time-lapse imaging of cytokinesis proteins in dividing germ cells in the Drosophila male germline, we previously showed that ring canal formation occurs via dramatic reorganization of the germ cell midbody – a structure classically associated with its function in recruiting abscission-regulating proteins in complete cytokinesis. However, unlike canonical midbodies, the germ cell midbody is unusually large, short-lived, and rapidly reorganizes to form a nascent ring canal. We identified a component necessary for germ cell midbody reorganization, the mitotic kinase and midbody organizing protein Citron kinase/Drosophila Sticky. Our recent structure-function analyses have revealed a role for the Citron-Nik1 Homology (CNH) Domain as over-expression of a *sticky-ΔCNH::GFP* cDNA construct dominantly affects ring canal formation. The CNH domain was previously shown to be necessary for Sticky function in completely dividing S2 cells[1], suggesting that it may function analogously during the incomplete divisions that generate ring canals. As Sticky contributes to the maintenance of RhoA at cleavage furrows in completely dividing cells, we examined the localization of RhoA with the RhoA sensor (Anillin Rho Binding Domain::GFP) and found evidence that RhoA is localized to ring canals throughout spermatogenesis. Furthermore, our preliminary results show that over-expression of dominant negative, but not constitutively active, RhoA in an otherwise wild-type background results in smaller ring canals on average, suggesting that RhoA-GTP may play a role in ring canal formation. To identify additional ring canal promoting factors, we performed a candidate RNAi screen targeting mitotic kinases and phosphatases, as well as enzymes with important roles in ubiquitin homeostasis. We have identified several genes, some of which are uncharacterized, that are required for proper ring canal architecture and/or size suggesting an important role for post-translational modifications of germ cell midbody proteins during ring canal biogenesis.

1. Bassi, Z.I., Verbrugghe, K.J., Capalbo, L., Gregory, S., Montembault, E., Glover, D.M., and D'Avino, P.P. (2011). Sticky/Citron kinase maintains proper RhoA localization at the cleavage site during cytokinesis. J. Cell Biol. *195*, 595–603. 10.1083/JCB.201105136.

366 **TGF-β Ligand Cross-Subfamily Interactions in the Response of *Caenorhabditis elegans* to Bacterial Pathogen** Emma Ciccarelli[1], Zachary Wing[2], Katerina Yamamoto[1], Cathy Savage-Dunn[1] [1]Queens College and the Graduate Center, CUNY, [2]Queens College, CUNY

Transforming Growth Factor-β (TGF-β) secreted signaling ligands play critical roles in a range of biological functions and their dysfunction in humans is associated with diseases including cancer, cardiovascular disease, and reproductive disorders. These ligands are divided into bone morphogenetic protein (BMP) and TGF-β/Activin subfamilies that predominantly signal through different signaling pathways. Many TGF-β ligands have context-dependent or concentration-dependent functions. While the core components of TGF-β signaling pathways have been identified for more than 25 years, how these pathways produce context-dependent outcomes remains poorly understood. In the nematode *Caenorhabditis elegans,* the BMP ligand DBL-1 is required for innate immunity. We tested the roles of all five *C. elegans* TGF-β ligands in survival on the pathogenic bacterium *P. luminescens.* Mutants for each of the five ligands had reduced survival, with loss of TIG-2 and TIG-3 showing the most severe defects. Furthermore, *tig3; tig-2* double mutants had a survival rate indistinguishable from each of the single mutants, suggesting that they function together in pathogen response. Joint action of TIG-2 and TIG-3 could be evidence of TIG-2/TIG-3 heterodimers, or the ligands could act together through a receptor clustering mechanism. To determine whether heterodimers of TIG-2 and TIG-3 are feasible, we performed structural modeling. Using the ColabFold implementation of AlphaFold, we generated strongly supported models for the TIG-2/TIG-3 heterodimer and for the TIG-2 homodimer. In contrast, the potential for TIG-3 to form homodimers was not well supported by ColabFold. The genetic evidence and structural modeling support the hypothesis that these ligands form a heterodimer, in spite of being from different sub-families (BMP and TGFβ/Activin). Heterodimers of BMP ligands are functionally required or outperform homodimers in a variety of physiological contexts, but cross-subfamily heterodimers have not, to our knowledge, been identified previously. Cross-subfamily interactions between TGF-β ligands may diversify biological outcomes, allowing differentiation of acute responses, such as host-pathogen response, from more ubiquitous signaling functions.

367 **Drosophila gut microbiome colonization specificity is driven by selective adhesion** Kevin Aumiller[1,2], Karina Gutierrez-Garcia[2], Ren Dodge[2], Sneha Agrawal[1], Ann Deng[1,2], Xincheng Yuan[1], William Ludington[1,2] [1]Johns Hopkins University, [2]Carnegie Institution of Washington

Animals form symbiotic relationships with resident gut microbiota that are both stable and host-specific. The current consensus in the field is that host specificity is achieved by environmental filtering, wherein the host gut provides a unique

set of growth conditions for symbiotic bacteria through metabolism and immunity. We propose a higher degree of biological sophistication, where specificity is further enhanced by specific receptor-ligand interactions that allow the host to recruit bacteria to spatially defined niches.

Lactobacilli are prevalent symbiotic bacteria that exhibit diverse host ranges that include mammals and insects such as *Drosophila melanogaster*. We isolated a strain of *Lactiplantibacillus plantarum* (LpWF) from wild *D. melanogaster* that establishes a spatial niche within the foregut. Using this system, we developed a model to identify the determinants of host-specific colonization through *in vitro* evolution of LpWF.

Through long-read sequencing, we identified a genomic island consisting of 27 genes that are deleted via homologous recombination during *in vitro* evolution, with these mutants exhibiting (I) a sharp decrease in colonization efficiency, (II) a lack of spatial specificity for the foregut niche and (III) an acute selective disadvantage when placed in competition with wild-type LpWF in the fly gut.

Phenotypic characterization of several evolved mutants revealed that a pair of serine rich repeat adhesins (SRRPs) encoded by the island drive selection for strong colonizers in the gut, but are rapidly lost when LpWF is evolved outside of the host environment.

Through biochemical characterization of recombinant SRRP binding domains, we found that these adhesins specifically bind to the luminal surface of the foregut. Ectopic expression and cell surface display of the binding domains in SRRP-deficient mutants and non-fly isolate *L. plantarum* strains demonstrated sufficiency of these proteins to promote attachment to the foregut, suggesting that adhesion plays a key role in specifying colonization of the commensal niche.

From our findings, we propose that ligands secreted into the gut epithelial barrier promote specificity of colonization by modulating bacterial adhesion. We aim to identify the *Drosophila*-expressed binding partners of the SRRPs to further investigate the mechanisms by which animals establish and maintain specific host-microbe associations.

368 **Metabolic consequences of chronic bacterial infection increase susceptibility to starvation in *Drosophila melanogaster*** Andrea M Darby[1,2], Scott Keith[1,2], Ananda A Kalukin[1], Brian P Lazzaro[1,2] [1]Entomology, Cornell University, [2]Cornell Institute of Host-Microbe Interactions and Disease

Infections induce systemic shifts in host lipid and carbohydrate metabolism, including reduction in triglyceride and glycogen stores to support the immune response. *Drosophila melanogaster* that survive systemic infection with the bacterium *Providencia rettgeri* sustain a chronic bacterial load, exhibit infection-induced gene expression of antimicrobial peptides (AMPs), and are more susceptible to starvation at 10-days post-infection. In this study, we asked whether increased starvation susceptibility in survivors is due in part to metabolic consequences of sustaining a chronic infection. Further, we asked whether severity of infection correlates with altered metabolic phenotypes that affect starvation survival. To address this question, we infected flies with pairs of high- and low-virulence strains of the Gram-negative bacteria *Serratia marcescens* and *P. rettgeri*, which are natural pathogens to *D. melanogaster* and establish a chronic infection by five days post-infection. Flies that survived infection were measured for bacterial load, AMP expression, starvation resistance, triglyceride and glycogen levels, and expression of genes involved in triglyceride and glycogen catabolism. We found that high-virulence bacterial strains established higher bacterial loads and increased starvation sensitivity. The high-virulence *P. rettgeri* infection resulted in increased induction of AMPs, however, there was no difference in AMP induction between high- and low-virulence *S. marcescens* strains. Susceptibility to starvation after high-virulence *S. marcescens* infection was associated with reduced triglyceride and glycogen stores and elevated expression of the lipase *brummer*, while high-virulence *P. rettgeri* infection only resulted in reduced glycogen stores. These results suggest that carrying a higher chronic bacterial load results in metabolic consequences that increase susceptibility to starvation. To further test whether carrying a higher chronic burden increases starvation sensitivity, we infected flies with a low or high dose for each low-virulence and high-virulence strain to generate a low and high chronic burden within each strain. For both *S. marcescens* strains, the higher dose resulted in higher bacterial load and increased sensitivity to starvation. Current experiments are in progress to test whether a higher dose of the *P. rettgeri* strains also results in higher bacterial load and increased starvation sensitivity, and to test within strains whether higher bacterial load correlates with depletion of metabolic stores.

369 **Deep homolog scanning of proteins involved in host-pathogen conflict** Meru Sadhu National Institutes of Health

The advent of deep mutational scanning, in which the effects of all possible single-amino acid changes are assayed in a protein of interest, has given researchers an exquisitely detailed view of the immediate evolutionary landscape available to a particular protein sequence. On the other hand, looking across species, the orthologs of a given protein explore even greater diversity. Studying species-scale functional diversity can help us understand how genetic differences between species underlie their

unique traits. We are using array-based oligonucleotide synthesis and yeast-based phenotypic screens to study the functional differences of hundreds to thousands of homologs of proteins of interest. I will discuss our work on proteins involved in pathogen defense to better understand the genetic basis of species' susceptibility to hosting particular pathogens. We typically focus on particular surfaces of host and pathogen proteins that interface in a manner that is critical for the success or failure of the infection, such as interaction surfaces in defense proteins, host receptors, and pathogen effectors. We then build genetic libraries to test the homologous surfaces from diverse sequences for their ability to mediate the interaction. We propose that deep homolog scanning will help expand our understanding of the compatibility between hosts and pathogens, and the molecular basis for that specificity.

370 **Parasite-driven mitochondrial remodeling reprograms the host metabolic state to support parasite propagation and vertical transmission** Shenlu Qin[1], Matthew Sieber[2] [1]Physiology, UTsouthwestern Medical Center, [2]Physiology, UT Southwestern Medical center

Interactions between microbes and their hosts are central to normal physiology and human disease. In particular, host-parasite interactions are known to play a role in gastrointestinal disorders, metabolic diseases, and even cancer, all of which are driven by defects in cellular metabolism and mitochondrial function. Despite this relationship between metabolic defects and parasite infection in human disease, very little is known about the mechanisms used by intracellular parasites and pathogens to reprogram host mitochondrial function. Our lab uses a multi-system approach featuring *Drosophila*, human cells, and mice, incorporating genetic and biochemistry techniques to systemically define the conserved mechanisms that drive mitochondrial remodeling in disease.

One of the major challenges in studying the metabolic relationships between host and intracellular parasites is that purely cell culture-based strategies create an artificial metabolic state in the host cell. In this study, we employed the intracellular bacteria known as *Wolbachia* and *Drosophila melanogaster* as a robust *in vivo* model to investigate the evolutionary conserved aspects of host-microbe metabolic interactions. We have discovered that *Wolbachia* infection blocks the reprogramming of mitochondrial metabolism during *Drosophila* oogenesis. Our data shows that *Wolbachia* alters host ovary mitochondria shape, activity, membrane potential, and movement during cellular quiescence. Furthermore, we found that *Wolbachia* physically interacts with host mitochondria and produces proteins that target host mitochondria to control host metabolic function. We have found these mechanisms create a metabolic state that supports parasite needs. Notably, we discovered that alteration in host NAD+ biosynthesis and the methionine cycle are required for *Wolbachia* parasitism and its vertical transmission to the next generation. We are currently examining mammalian host-parasite interactions to determine if mitochondrial remodeling redox metabolism represents a conserved node commonly tagged by parasites in models of human disease. Overall, this study provides a mechanistic platform to understand the remodeling of mitochondrial metabolism by intracellular parasites. This study also provides a framework to identify metabolic vulnerability caused by parasite infections that enhance disease progression.

371 **Alternative splicing of conserved lipase alters endocannabinoid production and host innate immune response upon bacterial infection** Francis RG Amrit[1], Julia A Loose[1], Laura Bahr[1], Abbe Vallejo[1], Ravi Sharma[2], Andreas Pfenning[3], Ramanathan Sowdhamini[4], Carles Sune[5], Carissa P Olsen[6], Hidehito Kuroyonagi[7], Arjumand Ghazi[1] [1]Pediatrics, University of Pittsburgh School of Medicine, [2]Amity Institute of Biotechnology, [3]Carnegie Mellon University, [4]National Centre for Biological Sciences, [5]LogoCab LogoIPBLN Instituto de Parasitología y Biomedicina, [6]Worcester Polytechnic Institute, [7]University of Ryukus

Lipids exert a profound influence on immune status providing bulk energy sources and mediating immune signaling. Indeed, host responses to microbiota and infection have immune and metabolic facets. But, mechanisms that govern immunometabolic remodeling are poorly understood and have been studied mostly in the context of transcriptional regulation. The role of post-transcriptional mechanisms such as Pre-mRNA splicing in host-bacteria conflict remains largely unknown. We have uncovered a novel, evolutionarily conserved and physiologically relevant mechanism by which alternative splicing of lipases alters endocannabinoid (eCB) production and shapes the host response to intestinal bacterial infection.

Previously, we identified the *Caenorhabditis elegans* protein, TCER-1, homolog of human splicing factor TCERG1, as a regulator of lipid remodeling that modulated longevity, and showed that it represses host immune response against multiple bacterial infections. We have now discovered that infection triggers global mRNA splicing changes, especially of lipid-metabolic genes controlled by TCER-1/TCERG1. TCER-1/TCERG1 controls the inclusion/exclusion of a single exon in DAGL-2, a diglyceride lipase responsible for biogenesis of 2 Arachidonyl Glycerol (2-AG), the most abundant mammalian eCB with key immunomodulatory roles. Our *in vivo* studies have revealed dynamic cell- and tissue- specific patterns of DAGL-2 alternative splicing upon infection. Importantly, DAGL-2 alternative splicing determines 2-AG levels and impacts the host response to bacterial infection. Computational structure-function modeling identified analogous exons in DAGLβ, the human homolog of DAGL-2. We found DAGLβ alternative splicing to be regulated by human TCERG1 and DAGLβ exhibits similar alternative splicing in peripheral

blood cells suggesting strong mechanistic conservation. Overall, this work establishes alternative splicing as a conserved regulatory feature of immunometabolism and host-bacteria conflict.

372 **The gut microbiome is a significant contributor to mouse model phenotypes contributing to a lack of reproducibility and masking of genetic drift** James Amos-Landgraf, Aaron Ericsson, Craig Franklin Veterinary Pathobiology, University of Missouri

The genetics community has long recognized that genetic background can modify phenotypes of animals. The reproducibility and translatability of genetically modified mouse models remain problematic for many models. Recent work by our group and others has highlighted that not only the host genome but also the host microbiome can significantly contribute to the phenotype but is often not controlled for between institutions, investigators, or experiments. We propose that a minimal characterization and reporting of the gut microbiome (GM) can enhance the rigor and reproducibility of mouse models. The Mutant Mouse Resource and Research Center at the University of Missouri has now examined six well-characterized mouse models of human disease and found quantitative differences in model phenotypes in animals harboring different vendor-derived specific pathogen-free (SPF) GMs. We have created CD-1 colonies that harbor the GMs of four major commercial vendors' SPF microbiomes that we use as surrogate dams for rederivation or transgenic mouse model production. While the resulting animals are SPF, 16s rDNA sequencing revealed unique taxa in each of the GMs and significant differences in relative abundances of various shared taxa. Using predictive algorithms and untargeted mass spectroscopy-based metabolomics we identified dramatic differences in the metabolic capacity between the most diverse and complex microbiome and the least. We examined phenotypes in the IL10$^{-/-}$, NOD/ShiLtJ, C57BL/6-*Apc*^{Min}, DSS-induced colitis, and infectious disease models, as well as the BTBR model of behavior, and found significant differences in phenotypes as determined by histology, quantitative tumor development, disease severity, and behavior tests. In one case, the influence of the microbiome masked genetic drift in the population and compromised the comparison of datasets. In addition to characterizing and controlling the microbiome, our group as well as others are also altering the mouse microbiome to enhance the translatability to human disease through human microbe transfer. Banking fecal samples prior to moving or rederiving animals as well as prior to, during, and at the completion of experiments can act as insurance if phenotype changes occur. This would allow ruling out the microbiome if it remains largely unchanged. We believe that simple characterization and documentation of the microbiome present during an experiment can provide valuable data to enhance the rigor and reproducibility of mouse models and we propose that minimal standards for reporting be adopted.

373 **Nurse cell lipid droplets regulate actin remodeling by multiple prostaglandin-dependent pathways** Michael Welte[1], Michelle S Giedt[2], Jonathon M Thomalla[1,3], Roger White[1], Tina L. Tootle[2] [1]University of Rochester, [2]Department of Biology, University of Iowa, [3]Cornell University

During oogenesis, LDs (lipid droplets) accumulate in developing oocytes from flies to humans. These LDs are a crucial energy source for the developing embryo, but it was unclear what roles - if any - they play during oogenesis. We recently discovered that LDs mediate a critical signaling function in *Drosophila* nurse cells: the LD-associated lipase ATGL/Brummer cleaves LD triglycerides to release arachidonic acid, the substrate for prostaglandin synthesis; prostaglandin signaling then regulates actin cytoskeletal remodeling to drive follicle morphogenesis. To identify additional components of this pathway, we examined flies mutant for Jabba, an LD protein particularly abundant in the female germ line and early embryo. We find that Jabba is indeed necessary for proper actin remodeling and acts in a prostaglandin-dependent manner. However, this pathway is independent of ATGL and substrate release from LDs. Rather, Jabba appears to act downstream of prostaglandins, as mutants unable to produce prostaglandins display defects in both Jabba localization and the relative expression of the different Jabba isoforms. In addition, the actin defects in these mutants are partially rescued by increased *Jabba* dosage. Intriguingly, we detect Jabba not only on mature LDs, but also enriched near the cell periphery, the site of both LD biogenesis and actin polymerization. This Jabba/actin co-enrichment is likely functionally important as disruption of F-actin with latrunculin disperses peripheral Jabba; in addition, Jabba overexpression in a wild-type background results in abnormally thick cortical actin and actin bundles. We propose that prostaglandin signaling regulates Jabba, which in turn directly or indirectly impacts actin remodeling necessary for follicle development. This work supports the emerging idea that LDs and their associated proteins play critical roles in controlling the actin cytoskeleton and can do so by distinct molecular mechanisms. Given the conservation of LDs, prostaglandin signaling, and actin remodeling in oocyte development, these findings have implications for fertility across organisms.

374 **ESCRTs mediate Notch signaling in the testis stem cell niche** Mara R Grace, Erika L Matunis Cell Biology, Johns Hopkins University

Stem cell niches are dynamic microenvironments that provide signals to ensure the maintenance and self-renewal of adult stem cell populations. Proper signaling dynamics within the niche are crucial to maintain homeostasis, while disruption

of this signaling can lead to tissue death or overgrowth. Although much is known about signaling from niche cells to stem cells, little is known about signaling in the opposite direction, from stem cells back to their niche. The testis stem cell niche of *Drosophila melanogaster* is an excellent model to investigate such signaling. Using this model, we have uncovered a role for the endosomal pathway in mediating signaling from somatic stem cells, the cell population that supports the germline, to niche cells. Endocytosis regulates a myriad of signaling pathways as well as cellular communication. The ESCRT complexes are involved in a variety of cellular processes, such as multivesicular body formation and particle budding, and are considered endocytic tumor suppressor genes due to their role in signal attenuation. Here, I show that knockdown of several different ESCRT members in somatic stem cells results in an enlarged niche, both in cell number and overall niche volume, thus suggesting that ESCRTs mediate signaling from somatic stem cells back to their niche to prevent niche hypertrophy. Additionally, niche cells, while normally quiescent, enter the cell cycle when ESCRT members are knocked down in somatic stem cells. While investigating signaling pathways that may be regulated by ESCRTs, I found that Notch activity is increased in somatic stem cells upon ESCRT knockdown. Furthermore, I have found that knockdown of Notch in somatic stem cells prevents niche overgrowth, whereas expression of activated Notch in somatic stem cells results in an enlarged niche, thus suggesting that Notch signaling mediates the niche hypertrophy caused by ESCRT loss. This leads to a model where loss of ESCRTs in somatic stem cells leads to an autonomous increase in Notch pathway activity which then non-autonomously triggers niche overgrowth. This work demonstrates an instance of stem cell to niche communication, and, as the previously quiescent niche cells are now entering the cell cycle, has implications for tumorigenesis.

375 **Distinct regulators of lipid asymmetry regulate extracellular vesicle budding** Lauren Pitts[1], Alissa Rumin[1], Tanner Chase White[1], Alexander Nguyen[1], Julia Frondoni[1], Dinah Loerke[2], Ann M Wehman[1] [1]Biological Sciences, University of Denver, [2]Physics & Astronomy, University of Denver

The P4-ATPase TAT-5 acts as a lipid flippase to maintain phosphatidylethanolamine (PE) asymmetry in the plasma membrane and inhibits extracellular vesicle (EV) release from the surface of *C. elegans* cells. TAT-5 is required for embryonic development and fertility, but how flippase activity regulates these processes was unclear.

To test how flippase activity regulates different processes, we generated mutations in the DGET motif of TAT-5 predicted to lead to a 3-fold (D to T) to complete (E to Q) loss in lipid transport. We discovered that *tat-5(E246Q)* mutants were sterile, while *tat-5(D244T)* mutants produced embryos that arrested during development. Using degron-based reporters and an automated EV detection algorithm, we found that EV release was increased >100-fold in both mutants and that phagocytosis was also disrupted. These data suggest that a low level of flippase activity can support fertility, while a higher level of flippase activity is required to inhibit EV release, allow phagocytosis, and carry out embryonic development.

As PE lipids are unlikely to be exposed in flippase mutants without the help of lipid scramblases that act as channels between leaflets of a membrane bilayer, we also investigated which scramblase proteins promote EV budding. Using a sensitized screen, we identified four scramblases where their knockdown leads to a 2-fold decrease in EV release from the plasma membrane. Intriguingly, these scramblases act at various membranes in cells, including the ER, intracellular vesicles, and the plasma membrane, suggesting that internal membrane asymmetry influences surface asymmetry.

As lipid localization can alter physical properties of the membrane, we also started determining the physical characteristics of the membrane that promote EV budding. Worms raised at 23˚C release significantly fewer EVs after a 4-hour shift to 20˚C and significantly more EVs after a 4-hour shift to 26˚C. These temperature shifts are consistent with changes in membrane fluidity altering plasma membrane budding and we are investigating this more directly using lipid probes. These studies on EV release are also likely to provide insight into other membrane budding and tubulation processes.

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376 **Lipids from a yeast-based diet mediate adaptation of *Drosophila melanogaster* to cold temperatures: a polyunsaturated plasmalogen tail.** Claudia Y Espinoza[1], Emily Behrman[2], Nan Chen[2], Daniel Milshteyn[3], Gudrun Ihrke[2], Jennifer Lippincott-Schwartz[2], Itay Budin[1] [1]Chemistry and Biochemistry, UCSD, [2]HHMI's Janelia Research Campus, [3]UCSD

Thermoadaptation is crucial for life to persist on our planet. Lipids are key molecular components for overcoming fluctuations of temperature, as they must be modified to maintain membrane fluidity and curvature as temperatures drop. Increasing the abundance of phospholipids with polyunsaturated chains (PUFAs) across cell membranes is a commonly observed strategy for animal cold adaptation. *Drosophila* cannot produce PUFAs *de novo* because they lack the required desaturase enzymes. It has thus been proposed that flies prefer plant-based diets in the cold, in contrast to yeast-based ones, as a dietary source of PUFAs. We collected wild *Drosophila melanogaster* during warm and cold periods of the year and found a predominance of associated PUFA-producing-yeast during colder months, suggesting yeast can also serve as a dietary source of PUFAs for flies. We found that the PUFA producing yeast *Lachancea kluyveri (L. k)*, prepared as a as laboratory diet, allows flies to maintain

activity down to 8°C. Flies fed with two *L. k.* strains that carry mutations in the desaturases *fad2* and *fad3,* and thus cannot produce any PUFAs, have lower activity, as do those fed non-PUFA producing yeast, like *Saccharomyces cerevisiae*. We also found that flies show egg laying and foraging preferences over PUFA-producing-yeast substrates at cool temperatures (18°C) that changes at warm temperatures (25°C). These processes depend on the olfactory system. GC-MS analysis identified isoamyl acetate as the main variant volatile in the WT and PUFA-mutant *L. kluyveri* strains, and behavioral assays showed that flies have a temperature-dependent feeding preference for the yeast-produced concentrations of isoamyl acetate. Lipidomic analysis identified the plasmalogen plasmenyl phosphatidylethanolamine, a structurally peculiar lipid containing a *sn*-1 vinyl ether linkage and *sn*-2 PUFA chain, to be differentially synthesized in flies fed with the different *L. k* diets. *D. melanogaster* null mutants for plasmalogen biosynthesis change the PUFA-dependent cold-activity phenotype. Finally, attraction towards isoamyl acetate is its affected in low plasmalogen mutants. These observations point to a complex symbiotic relationship between yeast and *Drosophila* that is influenced by PUFA exchange, volatile production, and ether lipid biosynthesis that allows *Drosophila* to adapt to cold temperatures

377 **Fascin controls nucleolar function and morphology during Drosophila oogenesis** Danielle E Talbot, Tina L Tootle Biology, University of Iowa

Prostaglandins (PGs) are locally acting lipid signaling molecules that regulate development, fertility, cardiovascular disease, and cancer. However, the underlying cellular mechanisms remain poorly understood. Using *Drosophila* oogenesis as a model, our lab has identified PGs as a novel regulator of the nucleolus. Loss of PGs results in a rounded nucleolar morphology, increased nucleolar transcription, and protein translation. These alterations to nucleolar structure and function are due to increased nuclear actin. Thus, PGs limit nuclear actin to control nucleolar function and structure. Here we address the question of how PGs regulate nuclear actin to control nucleolar functions. Actin binding proteins (ABPs), including the actin bundling protein Fascin, localize and function in the nucleus. As PGs regulate both the actin bundling functions and the nuclear localization of Fascin, and over-expression of Fascin in a PG synthesis mutant rescues nucleolar morphology, we hypothesize that PGs regulate Fascin to control nuclear actin and thereby, nucleolar function. We find that loss of Fascin results in a dispersed nucleolar morphology, decreases nucleolar transcription, and a loss of nuclear actin enrichment. This data leads to the model that Fascin promotes normal nuclear actin accumulation to maintain the appropriate level of nucleolar function. This model is at odds with Fascin's ability to rescue the loss of PGs, leading us to speculate this rescue is due to restoration of the actin cytoskeleton when PG signaling is lost. Together these data suggest Fascin has both an indirect (actin cytoskeleton) and direct role in regulating the accumulation of nuclear actin. Current work is focused on testing the indirect role of Fascin. By uncovering the mechanisms by which PGs and Fascin regulate the nucleolus, we hope to gain a greater understanding of how the dysregulation of these factors can disrupt development and fertility, and fuel disease pathogenesis.

378 **Oligomerization and feedback on membrane recruitment stabilize PAR-3 asymmetries in *C. elegans* zygotes.** Charles Lang[1,2], Alexander Anneken[3], Edwin Munro[2] [1]Molecular and Cellular Physiology, Stanford University, [2]Molecular Genetics and Cell Biology, University of Chicago, [3]University of Chicago

The PAR polarity network is a paradigmatic example of how systems of mutually antagonism interactions among peripheral membrane binding proteins allow them to form and maintain complementary polar domains in response to a transient polarizing cue. The oligomeric scaffolding protein PAR-3 has emerged as a keystone member of the PAR network in many different contexts. In early *C. elegans* embryos, PAR-3 is required for all other PAR asymmetries, and it can form stable unipolar asymmetries when its known inhibitors are absent and all other members of the PAR network are cytoplasmic or spatially uniform on the membrane. But how PAR-3 forms stable unipolar asymmetries absent mutual antagonism is unknown. Here we combine single particle analysis with quantitative modeling and experimental manipulations to determine how the dynamics of PAR-3 membrane binding, oligomerization and dissociation allow PAR-3 to maintain stable asymmetries in the one cell *C. elegans* embryo. We find that two forms of positive feedback contribute to sustaining PAR-3 asymmetries: First, a sharp size-dependent decrease in oligomer dissociation rates makes the effective dissociation rate of PAR-3 decrease sharply with its membrane density. Second, membrane-bound PAR-3 promotes additional binding of PAR-3 to the membrane. Through a combination of modeling and quantitative measurements, we show that these two feedback loops are sufficient to dynamically stabilize asymmetries of the magnitude observed in polarized *C. elegans* zygotes. These results establish a dynamic basis for stabilizing unipolar PAR-3 asymmetries and add to the growing body of evidence that point to a central role for oligomerization of peripheral membrane proteins in the establishment and maintenance of cell polarity.

379 **Lipase mediated gut-brain communication regulates insulin secretion in *Drosophila*** Kandahalli Venkataranganayaka Abhilasha[1], Alka Singh[2], Kathya R Acharya[3], Jairaj K Acharya[1], Usha Acharya[1] [1]Cancer and Developmental Biology Laboratory, National Cancer Institute, Frederick, [2]MCCB, UMass Chan Medical School, Worcester, [3]University of Cincinnati College of Medicine

Pancreatic beta cells synthesize and secrete insulin in response to elevated glucose to maintain blood glucose levels within the physiological range. Defective insulin secretion is causal in most forms of diabetes. While glucose is the primary stimulus for insulin secretion by the beta cells, it is known that lipids obtained from diet or generated intracellularly can amplify glucose-stimulated insulin secretion (GSIS). How the dietary lipid pool potentiates GSIS, the lipid signals and mechanisms involved in relaying the signals to pancreatic beta cells have not yet been well established. This is a challenging endeavor because maintenance of glucose homeostasis after a meal poses substantial complexity. It requires the coordination of digestion, absorption, relay of signals from other organs, and the final integration of these inputs at the beta cells to determine the extent of insulin release. Thus, inter-organ communication plays a vital role in glycemic control. *Drosophila* is increasingly being appreciated as a model to dissect mechanisms governing metabolism that involve inter-organ communication. Many metabolic pathways are conserved between flies and humans. This includes the insulin pathway and in *Drosophila*, there are eight insulin-like peptides, DILP1-8, of which DILP2 is most closely related to human insulin. DILP2 is synthesized and secreted from median neurosecretory cells called the insulin producing cells (IPCs) in the brain. Here, we show, a *Drosophila* secretory lipase, Vaha, is synthesized and secreted from the midgut in response to dietary fat. It moves to the brain, where it concentrates in the IPCs in a process requiring Lipid Transfer Particle, a *Drosophila* lipoprotein originating in the fat body. Our results demonstrate Vaha participates in fat amplified insulin secretion. Vaha mutant flies, flies with gut specific knockdown of Vaha or flies lacking Vaha lipase activity, all show decreased release of DILP2 from the IPCs upon feeding after fasting. Vaha mutant flies have reduced DILP2 in circulation and exhibit features of diabetes including hyperglycemia and hyperlipidemia. Our findings suggest Vaha functions as a diacylglycerol lipase in a gut-brain axis modulating DILP2 release to maintain glucose homeostasis in the adult fly.

380 **The stress response transcription factor Atf4 interacts with Ecdysone Receptor to maintain fat tissue homeostasis in *Drosophila*** Manuel H. Michaca[1], Lydia Grmai[1], Deepika Vasudevan[2] [1]Cell Biology, University of Pittsburgh, [2]University of Pittsburgh

The endoplasmic reticulum (ER) is the primary site of lipid synthesis and metabolism. Consequently, excess lipids or nutrient deprivation can cause highly metabolic cells, such as adipocytes and hepatocytes, to dysfunction. Such stresses trigger the ER stress response and induces the translation of the evolutionarily conserved ER stress response factor, Activating transcription factor 4 (Atf4). Atf4 restores cellular homeostasis through induction of stress-adaptive genes, or, alternatively, promotes apoptosis when homeostasis is irreparable. However, the molecular mechanism by which Atf4 specifies distinct suites of transcriptional targets under homeostasis versus ER stress conditions remains an open question. Here, I will use the *Drosophila melanogaster* third instar larva fat body as a platform to test the hypothesis that Atf4 interacts with nuclear receptors to switch between protective or deleterious transcriptional modes in metabolic tissues. My data show that homeostatic Atf4 transcriptional activity in the fat body is regulated by the nuclear hormone receptor, Ecdysone Receptor (EcR). Conversely, I also find that Atf4 is required for EcR transcriptional activity in the fat body. Loss of Atf4 has been demonstrated to result in overall reduced triacylglyceride content. Thus, I tested whether Atf4 and EcR coregulate lipid metabolism genes such as the triglyceride lipase, bmm. We found that loss of Atf4 or EcR results in increased expression of bmm in the fat body, suggesting that Atf4 and EcR repress *bmm* under homeostatic conditions. We next sought to examine if bmm is a direct transcriptional target of Atf4 and EcR. We next used the known Atf4 position weight matrix and found several Atf4 binding sites in the *bmm* genomic loci. Consistently with our *in vivo* data, we find that deleting Atf4 binding sites results in an enhancer-based reporter results in derepression of *bmm*. Surprisingly, this assay also revealed that Atf4 is required for induction of *bmm* under stress conditions. These data suggest that Atf4 has a dual role in *bmm* transcriptional regulation, as a repressor during homeostasis and an activator during stress. We are currently examining the role of EcR in Atf4-mediated regulation of bmm and extending this analysis to other lipid metabolism genes such as lsd1/2. Together, my data provide a mechanistic framework for understanding the dual protective and deleterious role of Atf4 in metabolic tissues.

381 **Glial KCNQ K^+ channels control neuronal output by regulating GABA release from glia in *C. elegans*** Bianca Graziano[1], Lei Wang[1], Olivia R. White[2], Daryn H. Kaplan[1], Jesus Fernandez-Abascal[2], Laura Bianchi[3] [1]University of Miami, [2]university of Miami, [3]Physiology and Biophysics, University of Miami

KCNQs (Kv 7) are voltage-gated K^+ channels expressed in the nervous system across species. In humans and mouse models, mutations in KCNQs have been linked to epilepsy and autism spectrum disorder (ADS). KCNQs are expressed both in neurons and glia. In neurons, KCNQs control neuronal excitability by setting the resting membrane potential and by regulating the firing rate. In glia though, the function of KCNQ channels is largely unknown. We used the model organism *C. elegans* to elucidate the function of KCNQ channels in glia and to determine their influence on neuronal function. In *C. elegans*, *kqt-1*, *kqt-2*, and *kqt-3* encode KCNQ channels homologs. Of these, *kqt-2* is expressed in the Amsh Sheath (Amsh) glia, a pair of glial cells that ensheath the dendrites of sensory neurons. Using behavioral assays, we found that *kqt-2* is needed in Amsh glia for response to the aversive odor octanol, suggesting that glial *kqt-2* is needed for neuronal output. Using in vivo Ca^{2+} imaging, we show that *kqt-2* is needed in glia for the activity of the L-type voltage gated Ca^{2+} channel *egl-19* and for Ca^{2+} transients in

response to octanol. Using the voltage sensor ASAP3, we show that *kqt-2* is required for setting the glial resting membrane potential and for voltage responses to octanol exposure. Using Ca^{2+} imaging, we show that loss of *kqt-2* in glia leads to the hyperactivity of the octanol sensitive neuron ASH, showing that neuronal hyperactivity is caused, not only by loss of KCNQ cannels in neurons, but also by their loss in glia. Finally, using optogenetics, genetic and pharmacological approaches we find that *kqt-2* is needed in glia for both tonic and phasic GABA release from glia. Importantly, all the *kqt-2* behavioral and cellular knockout phenotype are rescued by expression of the human KCNQ2 and KCNQ3 channels, supporting conservation of function across species. Finally, we show that loss of function (lof) and gain of function (gof) pathogenic KCNQ2 mutations reduce phasic and tonic GABA release respectively when expressed in Amsh glia. To conclude, our data point to the contribution of glial KCNQs to the expression of epilepsy and ASD phenotypes via reduction of GABA release from glia.

382 **A drug repurposing screen reveals novel biology and potential therapies for the rare disease DPAGT1-CDG** Hans M. Dalton, Alexys R. Berman, Kaylee A. Patterson, Sydney J. Peterson, Heather D. Evans, Clement Y. Chow Human Genetics, University of Utah

Glycosylation encompasses multiple biological pathways that affect essential sugar modifications of proteins. Mutations in glycosylation genes underlie Congenital Disorders of Glycosylation (CDGs) – ultra-rare disorders that cause seizures, developmental delay, and early mortality. Due to low CDG patient populations, it is challenging to conduct clinical trials for potential treatments. Consequently, there is a great need for alternative approaches to find therapies for these rare diseases. I use one such alternative – repurposing drugs by screening libraries of FDA/EMA-approved small molecules – to potentially speed up treatment development for CDG patients.

DPAGT1-CDG is caused by mutations in the gene *DPAGT1*, which encodes the first essential enzyme for N-linked glycosylation. Since total loss of *DPAGT1* is lethal, I created a model of DPAGT1-CDG in *Drosophila* via *DPAGT1* knockdown in the fly eye to cause a small, rough eye phenotype. This model can be screened for new therapies by quantitatively measuring improvements to its eye size. To find such therapies, I performed a drug repurposing screen by feeding flies 1,520 small molecules that are 98% FDA/EMA-approved. The top candidate drugs that rescue, resulting in a larger eye, were then validated by dose-response and genetic analyses.

I identified 40+ candidate drugs capable of rescuing the DPAGT1-CDG model eye size (Z-score ≥ 1.5). Top drug categories are dopamine receptor D2 (D2R) antagonists, acetylcholine-affecting drugs, COX inhibitors, and ion transporter-related drugs. Further genetic testing indicates that dopamine signaling is critical under *DPAGT1* loss. Overexpression of dopamine synthesis enzymes and knockdown of dopamine receptors rescued the *DPAGT1* model. This represents a potential new avenue for therapy - especially as seizures are common in DPAGT1-CDG patients. In addition, I genetically and pharmacologically validated rescue by an ion transporter inhibitor through its target *NKCC1*/*Ncc69*, as well as an acetylcholinesterase inhibitor through *ACHE*/*Ace* RNAi. All three pathways reveal novel biology related to *DPAGT1* mechanisms, and they may represent new therapeutic options for DPAGT1-CDG.

383 **Using *Drosophila* to understand the requirements of Alzheimer's risk genes in the central nervous system** Jennifer M Deger[1], Mingxue Gu[2], Shabab M Hannan[2], Oguz Kanca[2], Lindsey M Goodman[2], Ismael Al-Ramahi[2], Juan M Botas[2], Joshua Shulman[2], Hugo Bellen[2] [1]Neuroscience, Baylor College of Medicine, [2]Baylor College of Medicine

Alzheimer's disease (AD) risk is likely the amalgamation of many environmental and genetic factors. Thanks to data from genome-wide association studies (GWAS), we have new insights into genetic risk for AD. However, GWAS typically can't pinpoint which genes within susceptibility loci mediate association with disease risk. Furthermore, GWAS is limited by incomplete functional annotation of the genome. To address these limitations and extract actionable insights from AD GWAS, we have implemented a tiered, cross-species pipeline designed to dissect the functional genomic component of AD risk.

As of the summer of 2022, approximately 300 genes had been identified as AD GWAS loci. We prioritized these genes according to functional genomic evidence from human studies, i.e. the likelihood that the AD-associated variant alters the function and/or expression of the gene harboring it. In tandem, all 300 genes were included in an *in vivo* screen using *Drosophila* to find genetic modifiers of tau or amyloid-β (Aβ)-induced neurotoxicity. Elav-GAL4 driving UAS-human tau or Aβ induces a locomotor defect that worsens with age and can be enhanced or suppressed by RNAi-mediated knockdown of various genes. Together, our analyses identified 100 high-priority AD risk genes that were included in loss-of-function studies using *Drosophila* to characterize the requirements of these genes for central nervous system structure, function, and resilience to stress.

We generated Kozak or T2A-GAL4 alleles for 84 genes and crossed them to deficiencies. We determined that 40 high-priority AD genes were homozygous lethal in flies. For non-essential genes we used this same crossing strategy to create compound

heterozygous mutants to characterize loss-of-function phenotypes. For essential genes we crossed Kozak or T2A-GAL4 to two independent RNAi lines to create partial loss-of-function animals. These flies were aged and used for a battery of assays including histology to examine structural changes in the brain, electroretinograms to quantitatively measure neuronal function, and exposure to traumatic and heat stressors to assess neuronal resilience. Loss of approximately half of the genes tested thus far produce a defect in at least one assay. Our results provide insights into which genes with AD GWAS loci may be important for neuronal homeostasis. More broadly, these experiments allow us to assess potential functions for these genes and may help decipher the role of the genetic risk factors that have been identified for AD and other neurodegenerative diseases.

384 **Exploring glial induction of neuronal toxicity via the Draper pathway** Jayden Cyrus[1], Nina Sherwood[2], Emily Ozdowski[2] [1]Duke University, [2]Biology, Duke University

The *spastin* gene encodes a microtubule severing protein, and when mutated in humans leads to Autosomal Dominant Hereditary Spastic Paraplegia (AD-HSP), a neurodegenerative disease that impairs motor function in the legs. In *Drosophila*, *spastin* null larval neuromuscular junctions (NMJ) are characterized by a high synaptic bouton count with a "bunched" phenotype. In addition to neuronal proteins, glial proteins are also known to regulate synapses. One such protein is Pak3, a kinase that regulates actin polymerization and filopodial projections. Studies have shown that when *pak3* is deleted in *spastin* null *Drosophila*, neuronal structure and function are restored. While it is known that *pak3* deletion rescues the neurons by acting in subperineurial glia (SPG), the exact mechanism remains unclear. We hypothesize that Draper, an engulfment receptor in glia, may participate in this mechanism. Preliminary results indicate that, like *pak3* deletion, deleting *draper* and *spastin* rescues the *spastin* null phenotype, further demonstrating a requirement for glia in the pathology caused by loss of *spastin*. Additionally, although it is known that Draper functions in glia, the specific subtype of glia - wrapping, perineurial, or SPG - remains unknown. Therefore, my aim is to identify the specific subtype of glia in which Draper acts to cause the neuronal defects when *spastin* is lost. To do this, I will knock down Draper in a glial-specific manner using the UAS-GAL4 system to express draper-RNAi in a *spastin* null background. If a certain cell-type specific knockdown exhibits the same rescue as ubiquitous *draper* deletion, then Draper is likely working in these cells. My preliminary results indicate that Draper works in perineurial and wrapping glia, but not SPG. Interestingly, this implies that while Pak3 works in SPG, Draper acts in other glia, implicating four different cell types in this interaction: neurons, wrapping glia, perineurial glia, and SPG. Understanding the specific cell type(s) in which Draper acts will provide the first step in understanding how these proteins regulate neuronal structure and neuronal health, better our comprehension of how glia communicate with neurons and other glia, and shed light on the glial induction of neuronal toxicity.

385 **UNC-43/CaMKII regulates presynaptic assembly in *C. elegans*** Mizuki Kurashina, Kota Mizumoto Zoology, University of British Columbia

Neurons communicate via a specialized interface known as the synapse comprised of presynaptic and postsynaptic specializations. At the presynaptic specialization, the release of neurotransmitter is controlled by highly conserved proteins that reside at a protein-dense region called the active zone (AZ). AZ proteins control recruitment of neurotransmitter-containing synaptic vesicles (SVs) and their exocytosis for synaptic transmission. While the structural and functional components of the AZ are well characterized, less is known about how they assemble into a functional synapse. Here we found that *unc-43*, a sole ortholog of calcium/calmodulin-dependent protein kinase II (CaMKII), is integral for proper presynaptic assembly in *Caenorhabditis elegans*. To visualize synapses, we use a transgenic strain expressing GFP-tagged CLA-1, an AZ protein controlling SV clustering, and TdTomato-tagged RAB-3, a SV-associated small GTPase, In wild type animals, CLA-1 is localized as a single punctum at the tip of synaptic varicosity labeled with RAB-3. In *unc-43* loss-of-function (lof) mutants, we observed disorganized presynaptic structures characterized by an increased number of CLA-1 puncta with weaker fluorescence intensity, and diffuse localization of RAB-3 along the axon. This observation suggests that *unc-43* is required for proper presynaptic assembly. Consistently, *unc-43* gain-of-function (gof) mutants have 'overdeveloped synapses' with increased CLA-1 and RAB-3 fluorescence intensity. To determine whether *unc-43* controls the localization of other presynaptic proteins, we are examining the localization of endogenously tagged AZ proteins in *unc-43(lof)* mutants.

To examine the functional conservation between UNC-43 and human CaMKII in presynaptic assembly, we generated a *C. elegans* strain in which the endogenous *unc-43* locus was replaced with codon-optimized human CaMKIIA (*hCaMKIIA*). We found that *hCaMKIIA* fully replaces the functions of *unc-43* as *hCaMKIIA* animals exhibit wild type locomotion and have normal presynaptic structures judged by CLA-1 and RAB-3 localization. We then introduced recessive and dominant mutations that are found in the CaMKII genes of patients with intellectual disabilities. Indeed, these disease-associated mutations in hCaMKIIA resulted in presynaptic organization defects similar to those in the *unc-43(lof)* and *unc-43(gof)* mutants. These observations suggest the functional conservation between UNC-43 and human CaMKIIA in presynaptic assembly.

386 **SWI/SNF and PMK-1/p38 regulate the C-type lectin gene *clec-67* to modulate acute responses to alcohol** Laura Mathies[1], Tyler Crossen[1], Jonathan Lindsay[2], Andrew Hsiao[3], Andrew Davies[1], Jill Bettinger[1] [1]Pharmacology & Toxicology,

Virginia Commonwealth University, [2]Biology, Emory & Henry College, [3]Laboratoire MeLiS, UCBL - CNRS

Alcohol use disorder (AUD) is a significant societal problem, for which there are few pharmacological interventions. This is, in part, because of the complex pharmacology of ethanol. We use *C. elegans* to model the acute effects of ethanol on neuronal function to identify molecular targets of ethanol and determine the mechanisms underlying the development of tolerance to those effects. We have found that SWI/SNF genes are associated with alcohol dependence in human populations and are important for behavioral responses to ethanol in *C. elegans* (Mathies *et al.,* 2015). SWI/SNF chromatin remodeling complexes consist of multiple subunits that can be combined to generate functionally and molecularly distinct chromatin remodelers. Two major subfamilies of SWI/SNF are BAF (Brg1/Brm-associated factors) and PBAF (Polybromo-associated BAF). We found that different SWI/SNF complexes are required for two aspects of the acute response to ethanol: initial sensitivity and the development of acute functional tolerance (AFT). The BAF complex is required for normal initial sensitivity, while PBAF is required for AFT. A transcriptomic analysis identified 603 genes that are regulated by PBAF in adult neurons; innate immunity genes were overrepresented among these genes (Mathies *et al.,* 2020). We examined members of each innate immunity pathway to explore this overrepresentation. We found that the PMK-1/p38 mitogen-activated protein kinase (MAPK) pathway genes *tir-1, sek-1, nsy-1,* and *pmk-1* are all required for the development of AFT. PMK-1/p38 pathway genes are not among the SWI/SNF-regulated genes, and vice versa. Therefore, we sought to identify genes acting downstream of both pathways. A previous study identified genes regulated by *pmk-1* and *sek-1* (Troemel *et al.,* 2006). Nine genes are regulated in the same direction by *pmk-1, sek-1*, *swsn-1*, and *swsn-9*; these are candidate downstream mediators of the effect of SWI/SNF and PMK-1/p38 on AFT. We focus here on the C-type lectin gene *clec-67*, which has increased expression in PMK-1/p38 and SWI/SNF mutants. Loss-of-function mutations in *clec-67* do not alter acute ethanol responses, but they do suppress the AFT defect of both *pmk-1* and *swsn-9* mutants. In the nervous system, *clec-67* is expressed primarily in ADL sensory neurons (CeNGEN). We found that we can rescue *clec-67* mutants by expressing the gene specifically in ADL neurons. Together, our data support a model wherein SWI/SNF and PMK-1/p38 inhibit the expression of *clec-67* in ADL neurons to promote AFT. CLEC-67 is predicted to be a secreted protein, therefore ongoing experiments are aimed at identifying the molecular targets of CLEC-67. There is significant interest in the role of innate immune signaling in the development of AUD. Our studies in the simple model *C. elegans* can help to define the requirements of innate immunity genes in the nervous system response to alcohol.

387 **Neurogenomic profiles of schizophrenia and autism risk mutations defined through large-scale zebrafish phenotyping** Mary E.S. Capps-Lopez[1], Claire Conklin[2], Anna Moyer[1], Verdion Martina[2], Emma Torija[2], Ari Ginsparg[2], Morgan Klein[2], William Gannaway[2], Caleb Calhoun[2], Summer Thyme[1] [1]UMass Chan Medical School, [2]University of Alabama at Birmingham Medical School

Recent large-scale genomic studies have uncovered numerous genes linked to schizophrenia and autism. However, the specific impact of these genes on brain development and function remains unclear. Using optimized pipelines for high-throughput whole-brain activity mapping and behavioral profiling, we have established larval zebrafish phenotypes for 17 genes linked to autism, 6 to childhood-onset schizophrenia, and 16 to typical schizophrenia. The human mutations modeled in zebrafish are protein-truncating, amino acid substitutions, or copy number variants. The generated lines include five patient point mutations in the genes *deaf1*, *nsd2*, and *atp1a3*, which we compared to loss-of-function mutations to determine whether these protein-coding changes had a similar impact. Brain activity mapping revealed convergent phenotypes for several genes, as well as commonly affected brain areas. Behavioral changes included altered sensory processing and sleep behavior. For several lines, including loss-of-function and point mutation alleles, we performed RNA-sequencing at early developmental stages to define shared and distinct molecular mechanisms. Furthermore, beyond the larval screen, we discovered abnormal social interaction at 21 dpf for two of thirteen tested lines. Bulk RNA-sequencing of the adult brain for the *arid1b* line revealed disruptions to neuropeptide signaling, particularly to the relaxin-3 hormone previously found to be involved in social behavior in rodents. Ultimately, we expect in-depth studies of these zebrafish lines to nominate downstream targets of disease genes for rational drug development. In parallel to our zebrafish work, we have built a new computational method for *in silico* drug discovery. This method uses information from published structural data to guide predictions and has higher accuracy than commonly used docking methods. The exceptional sensitivity of larval whole-brain activity mapping, coupled with its potential throughput, makes this innovative approach well-suited for identifying molecules capable of mitigating mutant phenotypes. With these advancements, we aim to address the therapeutic challenges associated with these disorders, which currently lack effective treatments.

388 **Behavioral variation in psychomotor and incentive sensitization in Collaborative Cross mice** Lisa M Tarantino[1,2,3], Lauren S Bailey[3,4], Jared R Bagley[3,5], Ashley Charpentier[3,6], Troy Wilcox[3,6], Savanna Julian[3,6], Ashley Olson[3,6], Laura G Reinholdt[3,6], Leona H Gagnon[3,6], Vivek M Philip[3,6], Elissa J Chesler[3,6], James D Jentsch[3,5] [1]Department of Genetics, School of Medicine, University of North Carolina, [2]Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, University of North Carolina, [3]Center for Systems Neurogenetics of Addiction, The Jackson Laboratory, [4]Behavioral Neuroscience, Binghamton University, [5]Psychology, Binghamton University, [6]The Jackson Laboratory

Substance use disorders are a significant public health concern with devastating consequences for affected individuals, families and society as a whole. Cocaine use and overdose deaths involving cocaine have been steadily increasing since 2015. Despite the prevalence of cocaine use disorder (CUD), no approved therapies currently exist. The lack of effective therapies is due, in part, to our limited knowledge about the complex etiology of these disorders. Not everyone who uses cocaine will go on to develop a CUD suggesting that individual differences, including genetic factors, play a role in increasing risk. Inbred mice have been useful for studying genetic and environmental mechanisms that impact addiction relevant behaviors from initial exposure to changes in the rewarding and reinforcing effects of drugs of abuse. However, much of the existing data have been collected in very few strains, limiting both the generalizability and translational value of the work. The Collaborative Cross (CC) is a genetically heterogeneous reference population derived from 8 inbred mouse strains. The CC provides an excellent platform on which to study mechanisms by which exposure to drugs of abuse results in a transition to compulsive drug seeking behavior. We have characterized cocaine-induced locomotor sensitization in the CC. Locomotor sensitization is the phenomenon whereby repeated exposure to the same dose of a psychostimulant results in an augmented locomotor response. Locomotor sensitization is thought to reflect long-lasting neuroadaptations in the brain that contribute to drug seeking behavior. However, direct comparisons of locomotor sensitization and changes in incentive-motivational processes that imbue drugs and drug-related stimuli with enhanced salience (incentive sensitization) are largely missing in the literature. We have captured significant phenotypic diversity in locomotor sensitization in 50 CC strains. Our data present a unique opportunity to establish the relationship between psychomotor and incentive sensitization in this well-characterized, genetically diverse and tractable experimental population. In fact, data from a limited number of CC strains suggests that behavioral sensitization does predict incentive sensitization, but the relationship between the two behaviors differs based on genetic background. These data will generate hypotheses regarding genetic and non-genetic mechanisms of addiction that can be explored in more targeted studies.

389 **Chance and genetics shape the diversity of individual cognitive behavior in *Drosophila melanogaster*** Riddha Manna[1], Johanni Brea[2], Tomislav Štampar[3], Gonçalo Braga[2], Ivan Tomić[2], Ana Marija Jakšić[2] [1]School of Life Sciences, École Polytechnique Fédérale de Lausanne, [2]EPFL, [3]TS Robotics

Genotype x environment interactions have been well studied for many phenotypes, including simple behaviours such as turn-bias and locomotion. However, studying how such interactions affect complex behaviors like cognition and give rise to behavioral individuality is hard. This is because complex behaviours show high intra- and inter-individual variability that changes dynamically throughout an individual's life. To study genetic basis of individual cognitive ability, we need to measure this behavior in a large number of individuals in highly controlled environments. Such studies have been so far technologically unattainable and usually used non-multiplexed assays inevitably introduce an entanglement of experimental noise with biological individuality. As a result, genetic and environmental effects on cognitive individuality have so far been experimentally highly confounded and poorly understood. To surpass this challenge, we developed a high-throughput, multiplexed platform for screening visual learning in *Drosophila melanogaster*. We use the platform with meticulous transgenerational environmental control to measure genetic effects on individual learning ability in more than 100 wild-type genetic backgrounds represented by more than 6400 individuals. This experimental design allowed us to partition the effects of genotype and environment on individuality in learning outcomes and learned behaviours, including decision-making, change in lateralization, and modulation of activity. Our results demonstrate there is a significant genetic influence on mean learning ability but also on intra-genotype variability in learning. We find that the extent of the intra-genotype variability in individual learning outcomes is shaped through the genotype-specific interactions with minimal environmental perturbations. These micro-environments experienced during learning have been usually ignored or written off as noise. Using the behavioural platform, we can, however, capture and measure them to show that minimal environmental perturbations are sufficient to induce massive behavioural variation that interferes with identifying fine-grained genetic effects on learning. To probe the limits of micro-environmental effects on learning behaviours that are, for humans, impossible to track manually we have developed and validated a robotic platform that autonomously performs the experimental setup. The robot handles the flies and phenotypes learning behaviour with high precision and repeatability. We use the behaviour assays performed by the robot to demonstrate the severity of micro-environmental effects on estimates of complex cognitive behaviours and the futility of behavioural genetic determinism beyond extreme experimental control.

390 **Chromosomal Inversion In(2L)t modulates behavior in a temperature specific manner and could enable *Drosophila's* seasonal adaptation** Benedict Lenhart[1], Alan O Bergland[1], Joaquin CB Nunez[2], Lynsey Blevins[1], Ben Riley[1] [1]Biology, University of Virginia, [2]Biology, University of Vermont

Drosophila melanogaster populations adaptively track to seasonal patterns in selection pressure, leading to cyclical changes in genotype and phenotype. However, we lack an understanding of the functional genetic architecture of this rapid evolutionary change. Inversions are a plausible genetic mechanism of this rapid evolutionary change of multiple phenotypes because they can accumulate linked variation at many genes and are thus likely to be pleiotropic. We have recently put forth evidence that

the cosmopolitan inversion In(2L)t mediates seasonal adaptation through linkage of adaptive alleles. Frequency of In(2L)t changes seasonally, and regions within the inversion are highly enriched with seasonal loci identified from temperature-based environmental models. When we examined phenotypic data from the DGRP -a *Drosophila* genomic panel- presence of In(2l)t is significantly associated with changes in traits related to behavior, morphology, and metabolism. These In(2L)t associated phenotypic changes could be due to linkage between functional polymorphism or the pleiotropic effect of individual genes within In(2L)t. To validate the phenotypic effect of In(2l)t, we quantified the impact of inversion karyotype on behavior within different environments. Using an automated tracking system to stimulate and record behavior, we show that the presence of In(2l)t reduced duration of sleep and the intensity of startle response compared to non-inverted flies. These reductions are sex specific to females. We quantified behavior in different thermal environments, and showed the differences between inverted and non-inverted karyotype were minimal at colder temperatures, and larger at warmer temperatures. To elucidate whether the impacts of In(2L)t derived from linkage or pleiotropy, we used complementation tests between genetic deficiency lines to analyze the impact of different regions within In(2L)t, and identified a genomic region near the distal breakpoint that impacts behavior. Future work seeks to identify pleiotropic genes near the distal breakpoint and test which genes could be contributing to seasonal evolutionary changes in a suite of highly correlated traits.

391 **Glial expression of a hydroxysteroid dehydrogenase underlies natural variation in hitchhiking behavior** Heeseung Yang[1], Daehan Lee[2,3], Heekyeong Kim[4], Daniel E Cool[3], Young-ki Paik[4], Erik C Andersen[3,5], Junho Lee[1,6] [1]Department of Biological Sciences, Seoul National University, [2]Department of Biological Sciences, Sungkyunkwan University, [3]Department of Molecular Biosciences, Northwestern University, [4]Yonsei Proteome Research Center, Yonsei University, [5]Biology Department, Johns Hopkins University, [6]Research Institute of Basic Sciences, Seoul National University

Phoresy is a type of species interaction in which organisms disperse by attaching themselves to more mobile and usually larger animals. Hitchhiking species have evolved specific traits for physical contact and successful phoresy, but little is known regarding the evolution of such traits or the regulatory mechanisms involved. The nematode *Caenorhabditis elegans* displays a hitchhiking behavior, nictation, during its stress-induced developmental stage called dauer. Nictation involves the nematode standing and waving its body to enhance its chance of riding another organism. Dauer-specific nictation behavior has an important role in natural *C. elegans* populations, which experience boom-and-bust population dynamics. By hitchhiking onto carrier animals such as isopods and slugs, dauer larvae can disperse to a new favorable habitat where they can resume their reproductive growth. Here, we investigated the species-wide natural variation found in nictation behavior of 137 *C. elegans* strains sampled throughout the world. We performed genome-wide association mapping and found a quantitative trait locus (QTL) for natural variation in nictation behavior. We then attempted to specify the locus at the gene level and found that sequence variation in the promoter of a hydroxysteroid dehydrogenase, *nta-1*, underlie differences in nictation. The absence of *nta-1* expression in certain glial cells (GLRs), attributable to promoter variation, enhances nictation. This implies that the steroid metabolism in glial cells may regulate hitchhiking behavior. Furthermore, population genetic analysis of 550 strains and their geographic distribution suggest that two *nta-1* promoter haplotypes existed prior to the global expansion of *C. elegans* and have been maintained by long-term balancing selection. *nta-1* confers a trade-off between dispersal and post-dauer reproduction, and therefore provides the potential for balancing selection.

392 **Cell type differences underlying color vision diversification in *Heliconius* butterflies** Wei Lu[1], Nicholas VanKuren[2], Marcus Kronforst[2] [1]Ecology and Evolution, University of Chicago, [2]University of Chicago

Animals exhibit a rich diversity of behaviors and perceptual capacities to interact with the complex environment. With the benefit of minimizing pleiotropic effects, modifications on the peripheral sensory system appear to be a more frequent target for rapid behavioral adaptations. Mate preference is one such rapidly evolving adaptive behavior during the speciation process. Closely related but reproductively isolated species often evolve unique mating cues and the mate preference for conspecific mating cues.
Heliconius butterflies represent a classic example of adaptive radiation with extraordinary within species divergence and between species convergence in wing color pattern. These diverse wing colorations are also used as mating cues for male mate choice. Previous studies have shown that *Heliconius* males with divergent mate preference differ at multiple layers of the peripheral visual system, including photosensitive rhodopsin and inter-photoreceptor connections. However, we still do not know the transcriptomic changes underlying color vision diversification, which precludes a complete understanding from genetic variants to final behavior differences.
To better understand the regulatory network changes underlying color vision diversification, we conducted single-nuclei RNAseq (snRNAseq) and profiled more than 80,000 nuclei from both adult male and female *Heliconius* butterflies, spanning from polymorphic population to species of subgenus-level divergence (~14 Mya). We recovered all photoreceptor cell types including the most basally positioned butterfly R9 photoreceptor(homologous to Drosophila R8), which is usually inaccessible through antibody staining or in-vivo recording due to its position and small size. We also found that while most cell type abundances are similar across species and sex, a group of pigment cells is highly variable in males and the abundance is

associated with inter-photoreceptor connection variation. Surprisingly, this pigment cell type is marked by sens-2 expression, which is a candidate gene for male mate preference in *Heliconius*.
Overall, our snRNAseq results provide a comprehensive cell atlas for butterfly retina and enable us to identify cell type abundance and regulatory network changes in a group of butterflies undergoing rapid mate preference evolution. Compared to *Drosophila*, *Heliconius* butterflies have expanded color vision and our results provide insights into the conservation and evolvability of peripheral visual system.

393 **Convergent reduction of olfactory genes and olfactory bulb ratio in mammalian species at altitude** Allie M Graham[1], Elysia Seputra[2], Bogdan Kirilenko[3], Jason Presnell[1], Arianna Harrington[4], Chad Huff[5], Michael Hiller[3], Nathan Clark[2] [1]Human Genetics, University of Utah, [2]University of Pittsburgh, [3]LOEWE Centre for translational biodiversity genomics, [4]Southern Utah University, [5]University of Texas MD Anderson Cancer Center

Life at high altitude presents unique challenges. Species specialized for life there have evolved phenotypic changes in response, many of which are convergent among unrelated high-altitude lineages. We present evidence that mammalian species living at altitude repeatedly evolved a reduced palette of olfactory receptor genes (ORs) and apparently lower olfactory acuity. This convergent decrease of ~19% of ORs was discovered through an unbiased scan of pseudogenization events scored in the genomes of 27 high-altitude mammalian species as compared to 45 low-altitude species. Accordingly, reduction of olfaction is apparently a consistent response to long term colonization of high-altitude habitats. In contrast, receptor gene families involved in pheromone detection and taste were not lost. In agreement with OR gene loss, cranial endocast measurements show that the brains of high-altitude species have ~16% smaller olfactory bulbs relative to lowland relatives. Together, these repeated evolutionary outcomes suggest a general relaxation of constraint on olfaction at altitude, perhaps due to reduced odorant diversity in high-altitude niches or reduced effectiveness of mammalian olfactory physiology in thin, dry, or cold air.

394 **Sensory receptor expansion and neural accommodation between flies and butterflies** Ke Gao[1], Cara Genduso[2], Michael Perry[1] [1]Cell & Developmental Biology, University of California, San Diego, [2]New York University

The evolution of larger and more complex brains required existing neurons and neural circuits to accommodate new inputs. The genetic and developmental basis of how neural accommodation occurs is largely unknown. Relative to other insects such as Drosophila, butterflies have evolved more complex retinal mosaics and expanded color vision through the addition of a second R7-type photoreceptor per ommatidium (unit eye). We investigated the developmental basis of this change in the retina and how butterfly brains accommodate an expansion in sensory receptor input. We identified four differences in transcription factor expression during retina development between flies and butterflies and tested the genetic relationships between each. Modifying *Drosophila* retinas to have butterfly-like expression produces flies that recruit the “mystery cell” as a second R7, in the same position as in butterflies. These ommatidia have two R7s and each makes an independent stochastic choice, like butterflies, leading to three stochastically distributed ommatidial types instead of two. In *Drosophila*, the main target of each R7 is a single Dm8 neuron per retinotopic medulla column, and we asked how additional R7s impact Dm8 number and position. We show that in the presence of extra R7s, additional Dm8 cells are retained during development and that two Dm8s can now target the same medulla column. We propose a model in which an excess of Dm8 cells provided developmental flexibility that helped immediately accommodate an increase in sensory receptor input. Neurons like Dm8, which are made in excess during development to help accommodate population-level variability in input types, could provide increased developmental flexibility and enable broader neural evolution.

395 **Comparative population genomics reveals convergent signatures of adaptation in avian brood parasites** Ekaterina Osipova[1], Christopher Balakrishnan[2], Jeff DaCosta[3], Michael Sorenson[4], Wesley Warren[5], Tim Sackton[6] [1]Harvard University, [2]East Carolina University, [3]Boston College, [4]Boston University, [5]University of Missouri, [6]Harvard Univ

About 1% of all bird species are obligate brood parasites, in which where females lay their eggs in the nest of another species. This behavior has evolved independently seven times across the avian tree of life, and presents a particularly appealing opportunity to study the molecular correlates of a convergently evolved behavioral trait. To study patterns of recent adaptation associated with avian brood parasitism, we have generated population-scale resequencing data (n = 10-31 individuals per species) from five species of obligate brood parasite (representing three independent trait origins) along with nesting outgroups. These include the parasitic cuckoo finches and wydahs, the parasitic greater honeyguide, and the parasitic brown-headed cowbird. Using a comparative population genetics framework, we detect signatures of recent adaptation at multiple time scales in both nesting and parasitic species. Using McDonald–Kreitman tests, we find evidence for repeated adaption on proteins involved in spermatogenesis and sperm function in multiple parasitic clades, but not nesting sister clades. This is consistent with evidence for increased male-male competition in parasitic lineages associated with reduced parental investment. Furthermore, using methods to detect recent selective sweeps, we find evidence for putative positive selection on genes with neurological function in some parasitic lineages, and we assess evidence for a role of selection on noncoding

regions of the genome in transitions to brood parasitism.

396 **Identification of regulators of early CEPsh glia development in *Caenorhabditis elegans* by single-cell RNA sequencing** Simin Liu, Shai Shaham The Rockefeller University

Astrocytes are the most abundant glial cells in the vertebrate central nervous system, and play important roles in development, homeostasis, and plasticity of the brain. Astrocytes are a heterogeneous population with remarkable diversity in their morphology, molecular signatures, and physiological functions. While some transcription factors and signaling pathways have been implicated in astrogliogenesis, the precise mechanisms governing the specification and generation of distinct astrocyte subtypes remain not fully understood. The *Caenorhabditis elegans* CEPsh glia resemble astrocytes in their elaborate shapes, development from radial-glia-like precursors, postembryonic transcriptomes, and physiological functions. Despite similarities in morphology and molecular signature, the four CEPsh glia of each animal undergo distinct developmental processes and serve varied physiological functions. Therefore, they provide an excellent model system for uncovering the elusive factors contributing to the generation of diverse astrocyte subtypes. Using single-cell RNA sequencing, we profiled the transcriptome of newly generated ventral CEPsh glia, their direct progenitor cells, and their close relatives, which include other neuronal and glial cell types. Differential gene expression analysis reveals early CEPsh glia-specific molecular signatures, as well as genes shared with another glial cell type. Through a functional RNAi screen, we identified transcription factors that regulate the expression of marker genes in CEPsh glia, implicating their involvement in the acquisition of CEPsh glia fate. Our study provides a comprehensive lineage- and time-resolved transcriptomic atlas of early developing ventral CEPsh glia and the close relative cell types around the period of their generation. Given the similarities between CEPsh glia and astrocytes, we are excited about the possibility that vertebrate homologs of CEPsh glia fate regulators we identify will also direct astroglia fate acquisition in mammals.

397 **The nonsense-mediated decay pathway for RNA surveillance is necessary for the complete hypoxia response by *C. elegans*** Calista Diehl, Robert Horvitz MIT/HHMI

All aerobic organisms require oxygen to survive and have developed adaptive mechanisms to sense and respond to conditions of hypoxia (low O_2). Hypoxia drives many physiological stress responses and is a prevalent feature in numerous human pathologies including cancer, myocardial infarctions, and ischemic stroke. The major hypoxia-response pathway is evolutionarily conserved from worms to mammals and is driven by the transcription factor HIF-1, which is negatively regulated by the O_2-dependent prolyl-hydroxylase EGL-9 (EGLN/PHD in mammals). In *C. elegans*, increased HIF-1 activity, caused by either hypoxia or an *egl-9(lf)* mutation, induces numerous adaptive responses, including the retention of eggs in the uterus (the Egl phenotype), decreases in locomotion and defecation rates, and increased resistance to both hypoxia and other stressors, such as ER stress, oxidative stress and bacterial infection. Despite substantial progress in understanding the EGL-9/HIF-1 pathway, much remains to be learned about downstream effectors of the EGL-9/HIF-1 pathway and how they mediate adaptive responses to hypoxia. In this study, we utilized the striking Egl phenotype of *egl-9(lf)* mutants to screen for suppressors of the hypoxic stress response. We discovered that loss-of-function mutations in the conserved nonsense-mediated decay (NMD) pathway suppress the *egl-9(lf)*-induced changes in egg laying, indicating that functional NMD is required for a complete hypoxia response. NMD, which was originally identified in yeast and worms, is an RNA surveillance mechanism that degrades aberrant mRNA transcripts with premature termination codons and maintains cellular homeostasis in response to transcript errors. We found that some, but not all, of other *hif-1*-dependent responses are affected by NMD-pathway mutations, indicating that NMD modulates specific aspects of the HIF-1-dependent hypoxia response. Overall, our findings show that the link between the hypoxia response and NMD extends beyond egg laying and likely reflects a major integration of these two important and evolutionarily conserved stress-response pathways. Moving forward, we plan to identify the population of transcripts that are degraded by NMD in hypoxic conditions, with the hope of determining which, if any, aberrant transcripts provide the link between NMD and the hypoxia response.

398 **A recessive-by-dominant Dobzhansky Muller incompatibility causes embryonic lethality in *Caenorhabditis* hybrids** Dongying XIE, Yiming Ma, Pohao Ye, Gefei Huang, Yiqing Liu, Tongwen Wen, Zhongying Zhao Hong Kong Baptist University

Postzygotic Hybrid incompatibility (HI), such as hybrid lethality, is pivotal in speciation because it precludes gene flow and creates reproductive barriers. HI is typically the result of a negatively epistatic interaction between at least two genes following hybridization, i.e., the so-called Bateson–Dobzhansky–Muller incompatibility (DMI). Despite tremendous efforts to understand the genetic mechanism of speciation over the past decades, only a handful of DMI genes have been molecularly identified across taxa, which inhibits the interpretation of the processes driving speciation. Using *C. briggsae* and its sibling species *C. nigoni* as a model, we previously identified tens of HI loci between the two and mapped a hybrid lethality locus to the right arm of *C. briggsae* ChrIV. With a combination of high-resolution genetic mapping, targeted mutagenesis, and

complementation tests, we established the molecular identity of the hybrid lethality locus, which fits well with a recessive-by-dominant type of DMI. The DMI involves a recessive allele *shls-1* (Species Hybrid Lethality Suppressor) in *C. briggsae* and a dominant allele *shel-1* (Species Hybrid Embryonic Lethality) in *C. nigoni*. We first found that *shls-1* encodes for an enzyme indispensable in a conserved biogenesis pathway, perturbation of which caused embryonic arrest in both nematodes. Hybrid lethality is suppressed when *Cni-shls-1* is present. However, its absence leads to complete hybrid embryonic arrests. This suggested a potential recessive deleterious interaction between *Cbr-shls-1* and a dominant *C. nigoni* allele, which is masked by *Cni-shls-1* in the hybrids. We subsequently mapped the dominant allele, *Cni-shel-1*, through genome-wide screening and found that it is also located on the *C. nigoni* ChrIV, in close proximity to a *C. nigoni*-specific *shls-1* paralog. The characterization of the molecular identity of *Cni-shel-1* is still ongoing. We proposed that the recessive-by-dominant DMI was potentially initiated by a gene duplication event, and subsequently manifested through species-specific evolution of *shls-1*, driven by complex genome rearrangements between the two nematode species. Our results will establish the first DMI gene pairs in *Caenorhabditis* interspecies hybrid and provide novel insights into how duplicated genes can invoke negative epistasis, interrupt crucial biological functions and ultimately reinforce the species boundary.

399 **All animals are equal, but some tetraploids are more equal than others** Mara Schvarzstein[1,2] [1]Biology, Brooklyn College at the City University of New York, [2]Biology & Biochemistry programs, The Graduate Center at The City University of New York

Polyploidization or whole genome doubling (WGD) is the process of inheriting more than the two copies of each chromosome inherited normally in diploid cells. WGD is central to normal development, repair and regeneration, adaptation and speciation, cancer development and evolution, and crop domestication. Tetraploidy is the most common type of WGD in nature and is found in more than 30% of cancers where it promotes oncogenesis, metastasis, and drug resistance. Our lab developed a strategy to rapidly generate multiple independent tetraploid *C. elegans* strains from nearly any diploid strain. To aid in establishing *C. elegans* as a model for polyploidization and its effects at the cellular, organ, and animal levels we have compared the physiology and development (e.g., intracellular, cellular, and organ scaling, as well as cell division dynamics, self-fertility, and developmental timing) between isogenic diploid and tetraploid strains. Here I report that we observe variability between animals in established tetraploid strains and that we observed differences between independently derived isogenic tetraploid strains. For instance, we have seen that isogenic tetraploid strains have diverse levels of self-fertility and effects in intracellular scaling, and we observe distinguishable developmental timing effects between non-isogenic tetraploid strains. Together, these findings are exciting because they suggest that each independent tetraploidization event produces similar, but not identical, tetraploids, and that evolution is observable in tetraploid strains in relatively short periods of time. However, it also highlights the need to use at least two independently derive isogenic tetraploid strains for studies using this model.

400 **Sexual dimorphisms in meiotic chromosome structures drive heat induced male infertility in *C. elegans* and *D. rerio*** Cori Cahoon, Colette Richter, Amelia Dayton, Diana Libuda University of Oregon

Many of the developmental processes critical for the genome stability of developing sperm and egg are directly altered by environmental stressors, such as temperature, toxins, and pH. In contrast to egg development, developing sperm are incredibly sensitive to changes in temperature with heat exposure strongly linked to genome instability, infertility, and cancer. Despite these detrimental consequences of heat on male reproductive health, the mechanisms behind heat-induced male infertility are unknown. Here we show in *Caenorhabditis elegans* and *Danio rerio* (zebrafish) that differences in the temperature sensitivity between sperm and egg development are due to sexual dimorphisms in the synaptonemal complex (SC), a conserved, meiosis-specific chromosome structure required for meiotic genome stability and fertility. We find that only the spermatocyte SC in both *C. elegans* and zebrafish is destabilized and fragmented following acute heat exposure. Concurrently, heat exposure also causes an increase in double strand DNA breaks in both *C. elegans* and zebrafish. To establish the sexually dimorphic features rendering the SC temperature sensitive, we developed novel imaging tools for sex comparative live imaging studies in *C. elegans* and identified several sexual dimorphisms in SC composition and dynamics without heat exposure. We also uncover functional roles for these sex-specific differences in SC composition where individual SC proteins regulate specific steps of meiotic DNA repair differently in each sex. Finally, we performed a forward genetic screen and identified mutants that both suppress and enhance the sex-specific mechanisms regulating heat sensitivity of the SC. Cytological analysis of these mutants suggest sexual dimorphisms in SC assembly and disassembly may contribute to the heat sensitivity of the complex. Together, these studies reveal the sexual dimorphisms within the SC and provide insights into how developing sperm and eggs adapted similar chromosome structures to differentially regulate and execute meiotic stress responses to temperature.

401 **Pervasive sublethal effects of agrochemicals as contributing factors to insect decline** Lautaro Gandara[1], Richard Jacoby[1], François Laurent[2], Nikolaos Vlachopoulos[1], Noa Borst[1], Matteo Spatuzzi[1], Gülina Ekmen[1], Clement Potel[1], Martin Garrido-Rodriguez[1], Antonia Böhmert[3], Natalia Misunou[1], Bartosz Bartmanski[1], Xueying Li[1], Dominik Kutra[1,1], Jean-Karim Hériché[1], Christian Tischer[1], Maria Zimmermann-Kogadeeva[1], Victoria Ingham[3], Mikhail Savitski[1], Jean-Baptiste Masson[2],

Michael Zimmermann[1], Justin Crocker[1] [1]EMBL, [2]Pasteur Institute, [3]Universitätsklinikum Heidelberg

Insect biomass is declining across the globe at an alarming rate. Climate change and the widespread use of pesticides have been hypothesized as two underlying drivers. However, the lack of systematic experimental studies across chemicals and species limits our causal understanding of this problem. Here, we employed a chemical library encompassing 1024 different molecules—including insecticides, herbicides, fungicides, and inhibitors of plant growth—to investigate how insect populations are affected by varying concentrations of pesticides, focusing on sublethal doses. Using a controlled laboratory pipeline for *Drosophila melanogaster*, we found that 40% of these chemicals affect the behavior of larvae at sublethal concentrations, and an even higher proportion compromises long-term survivability after acute exposure. Consistent with these results, we observed that exposure to chemicals at doses orders of magnitude below lethality induced widespread phosphorylation changes across the proteome. The effects of agrochemicals were amplified when the ambient temperature was increased by four degrees. We also tested the synergistic effects of multiple chemicals at doses found widely in nature and observed fitness-reducing changes in larval developmental time, behavior, and reproduction. Finally, we expanded our investigation to additional fly species, mosquitos, and butterflies and detected similar behavioral alterations triggered by pesticides at sublethal concentrations. Our results provide experimental evidence that strongly suggests sublethal doses of agrochemicals coupled with changes in environmental temperatures are significantly contributing to the global decline in insect populations. We anticipate that our assays can contribute to improving chemical safety assessment, better protect the environment, secure food supplies, and safeguard animal and human health, as well as understand our rapidly changing world.

402 **Single nuclei multiome ATAC and RNA sequencing reveals the molecular basis of thermal acclimation in *Drosophila melanogaster* embryos** Thomas S O›Leary[1], Emily E Mikucki[1], Sumaetee Tangwancharoen[1], Seth Frietze[2], Sara Helms Cahan[3], Brent L Lockwood[3] [1]University of Vermont, [2]Biomedical and Health Sciences, University of Vermont, [3]Biology, University of Vermont

Plasticity enables organisms to extend their tolerance limits. But often the physiological constraints that influence plastic responses are not well characterized, which hinders our ability to make predictions about tolerance limits. Here, we demonstrate that *Drosophila melanogaster* embryos undergo rapid thermal acclimation, on the timescale of hours, that extends their upper thermal limit. Given the speed of this response and the fact that early development is coordinated by changes in chromatin state and gene activation, we set out to characterize the molecular basis of embryonic thermal plasticity by performing single nuclei multiome ATAC and RNA sequencing on embryos that were acclimated to different temperatures. We found coordinated changes in chromatin state and the transcriptome in response to thermal acclimation. Overall, cool-acclimated embryos exhibited thermal compensation to the cold through increased chromatin accessibility and gene expression. Specifically, cold-acclimated embryos had higher chromatin accessibility of transcription factor binding motifs of global transcriptional activators, such as Zelda, and higher expression of genes encoding ribosomal proteins and enzymes involved in oxidative phosphorylation. Although most of these shifts were common to all cell types, many significant changes were cell type specific. For example, increased expression in the cold-acclimated embryos was particularly prominent in tracheal primordial cells, presumably to prepare for increased gas exchange to support a higher metabolic rate. On the other hand, we observed modestly higher expression of genes involved in cell migration and cell adhesion in warm-acclimated embryos, particularly in ventral nerve cord primordia, suggesting a potential role for locomotor and sensory development in embryonic heat tolerance. Overall, our results indicate that thermal plasticity is largely mediated by shifts in the epigenome and transcriptome that, at least in part, regulate the speed of development and that may impose metabolic costs that constrain upper thermal limits.

403 **Drivers of DNA Damage Tolerance in *Drosophila melanogaster*** Mitch McVey[1], Varandt Khodaverdian[2], Tokio Sano[2], Mai Tran[2], Lara Maggs[2], Alice Witsell[2], Nina Benites[2], Natalie Danzinger[2], Ana Dias[2], Rita Gyurko[2], Sofia Lombana Rengifo[2], Kian Sahani[2], Alexander Schmidt[2], Hanna Slutsky[2], Gina Tomarchio[2] [1]Biology, Tufts University, [2]Tufts University

DNA damage tolerance mechanisms allow for the completion of DNA replication in the presence of base and nucleotide damage. Experiments conducted largely in yeast and mammalian cells have identified translesion synthesis, repriming, and template switching as the major damage tolerance mechanisms. However, few studies have examined how multicellular organisms prioritize these strategies to ensure their survival, and the consequences that result when one or more are inactivated. To address this, we have conducted a systematic survey of Drosophila possessing mutations in genes involved in damage tolerance, and the response of these mutants to exogenous alkylation damage.

Based on results from genetic screens and reverse genetic analyses, we report five main findings: (1) homologous recombination/template switching is not the primary damage tolerance mechanism in Drosophila; (2) translesion synthesis, mediated largely by polymerases zeta and eta, is a central component of damage tolerance in rapidly dividing imaginal disc tissues; (3) Rev1 plays a critical role in tolerance by recruiting translesion polymerases and through its deoxycytidyl transferase

activity; (4) the SLX1/4 structure-specific endonuclease and the Drosophila ortholog of Ttf2 (transcription termination factor 2) become important for damage tolerance when translesion synthesis is impaired, and (5) collectively, these responses prevent the accumulation of double-strand breaks and catastrophic chromosomal shattering that lead to cell and organismal death. Together, our findings establish Drosophila as a versatile system in which to study tolerance strategies and discover novel mechanisms that promote organismal survival when replicating damaged DNA. Furthermore, they may provide insight into mechanisms that cancer cells use to survive conventional chemotherapeutic treatment.

404 **Asynchronous DNA synthesis establishes epigenetic asymmetry at sister centromeres and regulates both de-differentiation and re-differentiation in a stem cell lineage** Rajesh Ranjan[1], Xin Chen[2] [1]Biology, Howard Hughes Medical Institute, [2]Howard Hughes Medical Institute

Many stem cells utilize asymmetric cell division (ACD) to produce a self-renewed stem cell and a differentiating daughter cell. How non-genetic information could be inherited differentially to establish distinct cell fates is not well understood. We reported a series of spatiotemporally regulated events that ensure differential enrichment of the centromere-specifying component CENP-A between sister chromatids for their biased attachment and segregation during ACD of *Drosophila* male germline stem cells (GSCs). Our results provide the cellular basis for partitioning epigenetically distinct sister chromatids during stem cell ACDs.

However, the mechanisms that establish asymmetric sister centromeres between genetically identical sister chromatids is not well understood. Using Super-Resolution Chromatin Fiber (SRCF) assay, I found asymmetric recycling of pre-existing (old) CENP-A during DNA replication. Further, I found that CAL1, the chaperone for CENP-A, is required for asymmetric recycling of old CENP-A in S-phase GSCs. Compromising CAL1 not only abolishes CENP-A asymmetry between sister chromatids, but also results in mis-determination of germ cell fate, shown by both GSC loss and early-stage germline tumor phenotype. Furthermore, we found that DNA polymerases involved in lagging-strand synthesis, such as Polα and Polδ, are expressed at reduced levels in stem cells compared to non-stem cells in the same germline lineage. Interestingly, compromising either Polα or Polδ using heterozygotes is sufficient to recapitulate stem cell-specific asymmetric sister centromeres in symmetrically dividing progenitor cells. I hypothesize that the asynchrony between leading and lagging strand synthesis biases the pre-existing CENP-A recycling by the leading strand and helps establish asymmetric sister centromeres. To further understand the biological significance of induced epigenetic asymmetry in non-stem cells, I tested these cells for their de-differentiation and re-differentiation abilities *in vivo*. I found that under both physiological condition during aging or with genetic ablation of stem cells, the non-stem cells with enhanced epigenetic asymmetry behave more like *bona fide* stem cells, manifested by their more efficient restoration of stem cell-unique features and activities.

Together, these results illuminate the molecular mechanisms establishing asymmetric epigenetic information and highlight the important role of DNA replication in this process for cell fate determination in multicellular organisms.

405 **Transcriptional coupling of telomeric retrotransposons with the cell cycle** Mengmeng Liu[1], Xiao-Jun Xie[2], Xiao Li[3], Xingjie Ren[4], Jasmine Sun[1], Zhen Lin[5], Rajitha Udakara Sampath Hemba-Waduge[1], Jun-Yuan Ji[1] [1]Biochemistry and Molecular Biology, Tulane University School of Medicine, [2]Department of Molecular and Cellular Medicine, Texas A&M University Health Science Center, [3]Lewis-Sigler Institute of Integrative Genomics, Princeton University, [4]Department of Neurology, University of California, San Francisco, [5]Department of Pathology, Tulane University School of Medicine

Dipteran species employ a distinctive mechanism to safeguard chromosome ends, relying on the transposition of telomeric-specific retrotransposons (TRs), including *HeT-A*, *TART*, and *TAHRE* in *Drosophila*, rather than telomerases. While the role of TRs in generating tandem repeats at chromosome ends is established, the mechanism controlling TR transcription remained unclear. This study elucidates novel regulators of TR transcription and telomere length in *Drosophila*, identifying multiple subunits of the transcription cofactor Mediator complex, along with the transcriptional factors Scalloped (Sd) and E2F1-Dp. Depletion of multiple Mediator subunits, Dp, or Sd increases TR expression and telomere length, while overexpressing E2F1-Dp or depleting the E2F1 regulator Rbf1 stimulates TR transcription. Notably, Mediator and Sd affect TR expression through E2F1-Dp. Our CUT&RUN analysis revealed direct binding of CDK8, Dp, and Sd to telomeric repeats. These findings highlight the essential role of the Mediator complex in maintaining telomere homeostasis by regulating TR transcription through E2F1-Dp and Sd, revealing the intricate coupling of TR transcription with the host cell-cycle machinery for chromosome end protection and genomic stability during cell division. Additionally, this study reveals how *Drosophila* synchronizes the initial step in the TR life cycle with the cell cycle through factors such as E2F1-Dp to safeguard chromosome integrity.

406 **A sperm-killing selfish X chromosome in Drosophila exhibits female meiotic drive** Graeme L. Keais[1,2,3], Chadi M. Saad-Roy[4,5], Emmanuel Gonzalez[6], Ryan M.R. Gawryluk[1], Pauline van den Driessche[7], Benjamin Loppin[6], Steve J. Perlman[1] [1]Department of Biology, University of Victoria, [2]Department of Botany, University of British Columbia, [3]Biodiversity Research Centre, University of British Columbia, [4]Miller Institute for Basic Research in Science, University of California,

Berkeley, [5]Department of Integrative Biology, University of California Berkeley, [6]Laboratoire de Biologie et Modelisation de la Cellule, Ecole Normale Superieure de Lyon, [7]Department of Mathematics and Statistics, University of Victoria

Meiotic drivers are selfish genetic elements that bias gene transmission in their favor during meiosis or gamete development. These elements play a powerful role in centromere and genome evolution. Meiotic drive occurs through both males and females, but sex differences in gametogenesis circumscribe the mechanism of drive in the two sexes: Male meiotic drivers kill gametes that do not inherit them, while female meiotic drivers preferentially segregate to the egg cell during meiosis. We have discovered an X chromosome in *Drosophila testacea* that is capable of meiotic drive through both sexes. As previously reported, male carriers transmit the driving X to over 80% of offspring through a sperm-killing mechanism. We now report significant distortion of the driving X through females heterozygous for this chromosome. We find that both the adult offspring and embryos of heterozygous females inherit the driving X significantly more than the wild-type X. We also find that heterozygous females maintain normal fecundity, suggesting that distortion occurs during meiosis itself. To support this notion, we use microscopy to reveal that the driving X has undergone extensive elongation near the centromere, which may be related to the mechanism of drive in females. Male and female drive of this chromosome therefore appear to use different mechanisms. Overall, our results show that the driving X of *D. testacea* is not only a novel case of female meiotic drive, but the first example of a driving X capable of meiotic drive through both sexes since their discovery nearly 100 years ago and a striking example of collaboration between different meiotic drive elements. Meiotic drive through both sexes has major implications for the origin and evolutionary dynamics of the driving X in this species, and in other systems where female drive may be present but unnoticed.

407 **The synaptonemal complex plays multiple roles in establishing the recombination landscape across chromosomes** Katie Billmyre Genetics, University of Georgia

One of the top factors associated with aneuploidy is the disruption of meiotic crossing over. Interestingly, we know from extensive work in humans and model systems that meiotic segregation is not a uniform process for all chromosomes and instead a variety of mechanisms ensure segregation. However, the mechanisms underlying recombination and segregation of specific chromosomes are not understood. Here we use a model of chromosome-specific meiotic defects in *Drosophila melanogaster* to investigate the mechanisms that govern meiotic events on individual chromosomes. We find that small in-frame deletions in the transverse filament protein of the synaptonemal complex (SC), a key component of the meiotic machinery, result in fragmentation of the SC. These mutants also exhibit chromosome-specific defects where pairing and crossing over are lost on the X chromosome but not the autosomes. However, how the SC alters the recombination landscape on the X chromosome but not the autosomes is unclear. We used genomics to examine the relationship between the SC, double-strand breaks, and recombination on the X chromosome and the autosomes. Our sequencing results showed that in a partial loss of SC background, double-strand breaks can be repaired as non-crossover gene conversions, even when they are unable to be repaired as crossovers on the X chromosome. This work will help us better understand the mechanisms that regulate meiotic events on individual chromosomes to ensure proper inheritance.

408 **Mechanisms of ring chromosome lethality** Adam Lin, Mary Golic, Hunter Hill, Kate Lemons, Kent Golic School of Biological Sciences, University of Utah

We generated circular forms of sex chromosomes and autosomes in *Drosophila melanogaster*. Each of these ring chromosomes exhibits significant dominant zygotic lethality. In mitotic divisions the ring chromosomes show frequent misbehavior. Double bridges resulting from sister chromatid exchange and catenated sister rings were the most frequent anaphase aberrations. We also observed instances of mitotic nondisjunction, mitotic recombination between the ring and its homolog, and ring chromosome breakage. The frequent occurrence of catenated rings suggested that lethality could be ameliorated by overexpression of Topoisomerase II. Crossing ring males to females carrying additional copies of *Top2* significantly rescued ring-bearing offspring, while crossing to heterozygous *Top2*$^{+/-}$ mutant females resulted in complete lethality. We conclude that the normal level of maternal Top2 is not sufficient to regularly resolve catenated rings and is a major contributor to ring chromosome lethality. The DNA Damage Response also contributes to ring lethality: *chk2* and *p53* mutants show strong maternal rescue of ring-bearing progeny but with different phenotypes suggesting that Chk2 and P53 are detecting different aberrations. Finally, we saw many cases of homolog capture in mitosis, where a "sticky" connection between a ring chromosome and its linear homolog produced an anaphase bridge. We hypothesize that these represent remnants of somatic homolog pairing. This further suggests that somatic chromosome pairing is not merely homologs lying side by side, but that chromosome pairs are entwined by Top2-mediated passage of one chromosome through its homolog during interphase.

409 **Identification of UTR sequences involved in post-transcriptional gene regulation of vertebrate embryonic development** Jayesh Kumar Sundaram, Miler Lee Biological Sciences, University of Pittsburgh

The development of a fertilized egg into an adult organism with different specialized cell types requires precise gene regulation. After transcription, mRNA expression is influenced by factors that bind the untranslated regions (UTRs), which can influence stability, translation, and sub-cellular localization. These factors often bind to mRNAs by recognizing short regulatory sequences in the UTRs. To better understand post-transcriptional gene regulation during vertebrate development, we developed a novel method to perform unbiased identification of short UTR sequences that have dynamic prevalence in the transcriptome during development of six chordate species - zebrafish, medaka, Xenopus tropicalis, Xenopus laevis, axolotl, and amphioxus. We reasoned that developmental stage-specific changes in the transcriptome-wide abundance of a given sequence could reflect the activity of a stage-specific post-transcriptional regulator that binds to that sequence. For example, we observe a strong depletion of specific sequences corresponding to targets of the microRNA miR-430/miR-427 during gastrula stages, which is consistent with the known role of these microRNAs in regulating maternal mRNA clearance during the maternal-to-zygotic transition. We have also identified several other sequences of interest with significantly differential abundance during development, both species-specific and shared across vertebrates, which remain to be further characterized. Of note, across vertebrates, polypyrimidine sequences in the 5'UTR increase in abundance over time, which is consistent with the emergence of 5' TOP sequence-mediated regulation only later in development. We also found conserved enrichment of U-rich 3'UTR sequences in early stages in contrast to later stages, suggesting conserved roles for maternal mRNA regulation. The novel methods and findings in this study will help us better understand post-transcriptional gene regulation during embryonic development and beyond.

410 **Differential H3K4me2 distinguishes two enhancer classes at zygotic genome activation** Matthew D Hurton, Miler T Lee Biological Sciences, University of Pittsburgh

Early animal development requires dramatic gene regulatory changes, as an initially silent zygotic genome gains transcriptional competency and assumes developmental control from maternally deposited factors during the maternal-to-zygotic transition. In zebrafish embryos, maternal pluripotency factors Nanog, Pou5f3, and Sox19b (NPS) play critical roles in orchestrating zygotic genome activation, in part by opening condensed chromatin at distal regulatory elements, i.e. enhancers, and mediating the acquisition of activating histone modifications. However, some embryonic gene activation still occurs in the absence of NPS activity, suggesting the existence of other mechanisms regulating zygotic genome activation. To identify chromatin signatures of these unknown pathways, we profiled the histone modification landscape in zebrafish embryos using CUT&RUN, focusing on modifications associated with active regulatory sequence in other systems. Our regulatory map identified two subclasses of enhancers distinguished by presence or absence of H3K4me2. Further investigation revealed that enhancers lacking H3K4me2 require NPS for activation, while enhancers bearing H3K4me2 seem to be NPS-independent. Rather, these H3K4me2+ enhancers appear to be epigenetically bookmarked in a manner previously described for embryonic promoters. Together, these data implicate a parallel pathway for zygotic genome activation that does not depend on NPS pioneering, which likely has implications for understanding how pluripotency induction and transcriptome reprogramming are regulated in other species and contexts

411 **Conserved enhancers control notochord expression of vertebrate *Brachyury*** Cassie L. Kemmler[1], Jana Smolikova[2], Hannah R. Moran[1], Brandon J. Mannion[3], Dunja Knapp[4], Fabian Lim[5], Anna Czarkwiani[4], Viviana Hermosilla Aguayo[6], Vincent Rapp[7], Olivia E. Fitch[8], Seraina Bötschi[9], Licia Selleri[6], Emma Farley[5], Ingo Braasch[8], Maximina Yun[4], Axel Visel[3], Marco Osterwalder[7], Christian Mosimann[1], Zbynek Kozmik[2], Alexa Burger[1] [1]Pediatrics, University of Colorado, [2]Institute of Molecular Genetics of the ASCR, [3]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, [4]CRTD Center for Regenerative Therapies Dresden, Technische Universität Dresden, [5]Department of Medicine, University of California San Diego, [6]Department of Orofacial Sciences, University of California San Francisco, [7]Department for BioMedical Research (DBMR), University of Bern, [8]Department of Integrative Biology and Ecology, Michigan State University, [9]University of Zürich

Brachyury/T/TBXT genes encode T-box transcription factors that are critical regulators of notochord development, the defining feature of the chordate body plan. The notochord stabilizes the embryonic axis, secretes signaling molecules to guide neural tube patterning, and forms the template for spine development. Although anomalies in notochord patterning and spine formation are major contributors to disabilities worldwide, how *Brachyury* expression is controlled in normal and pathological conditions is unknown. The gene-regulatory enhancer elements that drive *Brachyury/T/TBXT* gene expression in the mammalian notochord have long remained elusive. Here, we combine genomic data from human chordoma tumors, evolutionary conservation analysis, cross-species transgenic reporter assays, and mouse knockouts to define three notochord-specific enhancer elements in the human *Brachyury/T/TBXTB* locus. Using transgenic assays in zebrafish, axolotl, and mouse, we establish the shared versus species-divergent activities of the three *Brachyury*-controlling notochord enhancers *T3*, *C*, and *I* in the human, mouse, and marsupial genomes. Acting as Brachyury-responsive, auto-regulatory shadow enhancers, deletion of all three elements in mouse abolishes Brachyury/T/TBXTB expression selectively in the notochord. We document enhancer dose-dependent trunk and neural tube defects downstream of Brachyury/T activity without impact on gastrulation

or tailbud formation. Surprisingly, the three *Brachyury/T/TBXTB*-driving notochord enhancers are conserved beyond mammals in the *brachyury*/*tbxtb* loci of fishes, dating their origin to the last common jawed vertebrate ancestor. Our data define an evolutionary ancient, essential enhancer combination dedicated to notochord control of *Brachyury/T/TBXTB* that advances our understanding of how this central transcriptional regulator is controlled in development and disease. Supported by the NIH, NSF, CHCO Foundation, and CU Anschutz.

412 **Decoding mitochondrial-nuclear interactions: Unraveling mechanisms of postzygotic isolation in Danionin fish species** Trevor J Chamberlain, Francisco Pelegri The Laboratory of Genetics, UW-Madison

Barriers to successful interbreeding arise as mechanisms of selection act on diverging species. One possible barrier is postzygotic isolation, in which mating and fertilization occur but embryos remain inviable. Members of the cyprinid subfamily Danioninae, which includes zebrafish, comprise a model phylogeny for studying biological mechanisms including postzygotic isolation by examining hybrid and nucleocytoplasmic hybrid (cybrid) embryonic development in a well understood developmental genetics framework. A suspected source of postzygotic species isolation lies in incompatibilities between the mitochondrial genome (mtDNA) of one species and the nuclear genome of another. To begin to study mito-nuclear incompatibilities, we sequenced mtDNA from zebrafish (*Danio rerio*) and related species *D. aesculapii*, *D. kyathit*, *D. nigrofasciatus*, *D. tinwini*, *D. albolineatus*, *D. margaritatus*, *D. erythromicron*, *D. choprae*, *Devario sondhii*, *D. aequipinnatus*, and *Microdevario kubotai*, assembled their mitochondrial genomes, and identified single amino acid variants (SAVs) among mitochondrial proteins. We performed analyses which showed episodic diversifying selection in *Danio albolineatus* mitochondrial respiratory complex I, and in complex IV proteins in species belonging to *Devario* and *Microdevario* genera. We also examined mitochondrial proteins for increased rates of amino acid divergence above neutral and found sites that experience positive selection, suggesting functional change. Leveraging the high degree of mitochondrial protein conservation among vertebrates, we then used 3D protein modeling to identify interacting nuclear-encoded gene candidates for further functional variant and positive selection analyses. These studies will determine co-evolving mitochondrial and nuclear genes that underly mechanisms of species isolation and provide targets for mitochondrial fitness assays in hybrids and cybrids.

413 **Zebrafish scales: a new model of skin appendage evolution and development** Andrew J Aman, David M Parichy Biology, University of Virginia

Vertebrate skin is endowed with a variety of skin appendages, a morphologically diverse class of protuberances and invaginations that includes teeth, hairs and glands in mammals, feathers in birds, keratinous scales in squamate reptiles, and calcified scales in non-tetrapod fishes. Did skin appendages evolve multiple times or do all skin appendages share common ancestry? Evolutionary relationships among these diverse appendages are contentious because of widely varying morphology, histology and extracellular matrix composition. Using developmental genetics, live imaging, and single cell transcriptomics, we have found striking similarities in the signaling networks that regulate zebrafish scale development and development of terrestrial skin appendages. Additionally, we found that zebrafish scale matrix is deposited by an epithelium that produces an enamel-like matrix and condensed mesenchyme that produces an elasmoid matrix that is distinct from other vertebrate mineralized tissues such as dentin or bone. Together, our work suggests a single, common origin of all vertebrate skin appendages and establishes zebrafish skin as a study system that will prove useful for discovering general mechanisms underlying skin appendage patterning and morphogenesis.

414 **Unraveling the role of evolutionary capacitors that obscure cryptic genetic variants in fish** Hannah A Grunwald[1,2], Matthew Harris[1,2], Clifford J Tabin[1] [1]Genetics, Harvard Medical School, [2]Orthopedic Surgery Research, Boston Children's Hospital

Imagine a school of fish—a group of extremely similar individuals, all the product of a large, outbred population. These individuals, despite phenotypic homogeneity, harbor extensive genetic variation. Why is this variation not apparent? Why isn't there a 1:1 relationship between genotype and phenotype? This disconnect has potentially profound consequences in the studies of ecology, evolution, and human health. It has been proposed that cells can use molecular "capacitors" to buffer cryptic genetic variation such that the phenotypic outcome of these variants is obscured. The most famous of these is Hsp90, a chaperone that stabilizes and aids in protein maturation for a diverse array of kinases, chromatin remodelers, and signal transducers. Inhibition of Hsp90 is sufficient to reveal cryptic variants in many animals and plants, however the specific mechanism(s) by which this buffering occurs, the extent of buffered systems, and the impact on evolution remains to be elucidated.

Hsp90-buffered variants are thought to play a crucial role in eye loss during the evolution of the blind Mexican cavefish, *Astyanax mexicanus*, and it was previously shown that inhibition of Hsp90 in these fish produces individuals with

a wider range of eye-sizes than untreated siblings. However, it has not been determined which specific genetic variants are responsible for changes in eye-size, and the feasibility of Hsp90 capacitance to accelerate or materially impact evolution has not yet been investigated. By combining datasets of Hsp90 clients with genes associated with eye-loss in existing QTL studies in *Astyanax*, we have identified a short list of 19 candidate genes that may explain Hsp90-dependent eye-loss in these fish. Using a variety of genetic tools in the zebrafish, including CRISPR knock-out and knock-in models and transgenic expression of dominant negative Hsp90 alleles, we are able to model and perturb Hsp90 buffering to investigate chaperone role in eye development and determine which specific Hsp90 clients may have played a role in *Astyanax* evolution.

415 **Functional validation of the albino *OCA2*^{NW273KV} polymorphism identified in a Caribbean Native American population in zebrafish** Thaddeus Harbaugh, Kathryn Early, Jessica Christ, Victor Canfield, Keith Cheng, Khai Ang Pathology, Penn State Hershey College of Medicine

Our interest in the genetic determinants of major population differences in human skin pigmentation led us to explore the contributions of genetic ancestry to human light skin color in a mixed Caribbean population of primarily Native American and African genetic ancestry residing in the Kalinago Territory of the Commonwealth of Dominica. Skin pigmentation phenotype measured by reflectance spectroscopy and expressed as the Melanin Index (MI) ranged from 20 to 80 units, averaging 45.8. Three albino individuals with light skin, hair, and eye color, and nystagmus were identified during fieldwork. Exome sequencing of an albino individual and an obligate carrier revealed a 4bp inversion, *NW273KV,* in the *OCA2* gene in all three albino individuals; these mutations were heterozygous in all obligate carriers. A second mutation in the *OCA2* gene, *R305W*, was also found in all three albino individuals. However, this was not the causative variant as two non-albino individuals were also found to be homozygous for this mutation. In further support of this conclusion, one individual who was homozygous for *R305W* but homozygous ancestral for *NW273KV* had an MI of 72, which was among the darkest in the entire population. The MI values of the three albino individuals were 20.7, 22.4, and 23.8, which were the lowest values among the Kalinago. The *NW273KV* polymorphism had a single allele effect size of -8 melanin units. The zebrafish (*Danio rerio*) model organism is ideal for the study of human pigmentation disease phenotypes given the conservation in function between species. Here, we used CRISPR-Cas9 to knock-in the *OCA2*^{NW273KV} polymorphism into a zebrafish model to functionally validate the albino allele. Wild-type zebrafish of the AB strain were crossed to produce embryos for microinjection of the CRISPR-Cas9 knock-in construct. These embryos were injected at the single-cell stage and raised to adulthood as the F_0 generation. Preliminary confirmation of the desired knock-in was qualitatively verified through the appearance of a mosaic albinism phenotype. We also performed allele-specific PCR to confirm successful knock-in of the *OCA2* allele. F_0 fish were then crossed, producing F_1 offspring that displayed a total loss of pigment. These results validate *OCA2*^{NW273KV} as the polymorphism responsible for albinism in the Kalinago population and highlight the value of zebrafish as a model for testing pigment phenotypes of human pigmentation alleles.

416 **Genomic factors shape metabolic niche breadth across an entire subphylum** Chris Todd Hittinger[1], Dana A. Opulente[1], Abigail Leavitt LaBella[2], Marie-Claire Harrison[2], John F. Wolters[1], Xiaofan Zhou[2], Xing-Xing Shen[2], Marizeth Groenewald[3], Antonis Rokas[2], Y1000+ Project Consortium[1] [1]University of Wisconsin-Madison, [2]Vanderbilt University, [3]Westerdijk Fungal Biodiversity Institute

Organisms, including yeasts, vary greatly in ecological niche breadth, from quite narrow (specialists) to quite broad (generalists). Paradigms proposed to explain this variation invoke either trade-offs between performance efficiency and breadth or underlying extrinsic or intrinsic factors. Here, we generated and assembled genomic (1,154 yeast strains from 1,051 species), metabolic (quantitative growth measurements in 24 conditions), and ecological (using a hierarchical environmental ontology) data from essentially all known species of the ancient yeast subphylum Saccharomycotina to examine niche breadth evolution. Using evolutionary genomic, machine learning, and network analyses, we found that large interspecific differences in carbon breadth stem from intrinsic differences in genes encoding specific metabolic pathways, but we found limited evidence for trade-offs or extrinsic factors. These comprehensive data argue that intrinsic factors drive yeast niche breadth variation. This unprecedented genomic, metabolic, and ecological dataset shows how metabolic diversity and niche breadth are encoded in yeast genomes and how these traits have evolved during more than 400 million years of yeast evolution. A preprint is available at https://www.biorxiv.org/content/10.1101/2023.06.19.545611.

417 **Genetic network rewiring between distantly related eukaryotes** Vanessa Pereira[1], Rohan Dandage[1], Elena Kuzmin[1,2] [1]Department of Biology, Centre for Applied Synthetic Biology, Centre for Structural and Functional Genomics, Concordia University, [2]Department of Human Genetics, Rosalind & Morris Goodman Cancer Institute, McGill University

Synthetic lethality represents an extreme example of a genetic interaction that occurs when a combination of mutations in different genes results in lethality, which would not be expected from the combined effects of individual viable single mutants. The extent of genetic interaction network conservation differs from genome sequence conservation between species. Two

distantly yeast species, *S. cerevisiae* and *S. pombe*, diverged ~500 Mya and despite 75% genome conservation, they display 29% genetic interaction network conservation. Other distantly related eukaryotes such as *C. elegans* and *H. sapiens* diverged ~600 Mya. Here, we investigate genetic network rewiring by studying the genetic interactions that underlie conditional essentiality of single mutants between *S. cerevisiae, S. pombe, C. elegans*, and *H. sapiens*, whereby a gene is essential (ES) in one species but nonessential (NES) in another. We identified 2853 *S. cerevisiae – S. pombe* orthologs, where ~15% are conditional ES. From 269 conditional NES *S. cerevisiae* genes (*S. pombe* ES*, S. cerevisiae* NES), we identified 124 cases which are rewired by synthetic lethal digenic interactions that modify conditional NES single mutants to synthetic lethal double mutants. Genetic interaction degree and phenotype rate of rewired conditional NES genes are higher than conserved NES genes suggesting that they are central on these networks. However, the lower phenotype rate of rewired relative to nonrewired conditional NES suggests that they are less pleiotropic. This finding is consistent with conditional NES genes being rewired by few synthetic lethal interactions, with most interactors modifying one conditional NES gene. To further understand the functional relationship between conditional NES genes and their rewiring synthetic lethal interactions, we overlapped them with common functional standards and found that they were co-expressed, co-localized, co-annotated, shared protein-protein interactions and showed similar phenotypic profiles suggesting that genetic rewiring of conditional NES genes is local. We extend these findings to *C. elegans* and *H. sapiens,* where ~21% of *H. sapiens – S. cerevisiae* orthologs, ~23% of *C. elegans – S. cerevisiae* orthologs, and ~14% *H. sapiens – C. elegans* orthologs are conditional ES. Mapping how conditional ES genes are rewired by genetic interactions will provide insight into genetic network rewiring and should reveal principles of genetic network conservation.

418 **Spindle architecture constrains karyotype in budding yeast** Jana Helsen[1], Md Hashim Rez[2], Gavin Sherlock[3], Gautam Dey[1] [1]EMBL, [2]Jawaharlal Nehru Centre for Advanced Scientific Research, [3]Stanford University

The eukaryotic cell division machinery must rapidly and reproducibly duplicate and partition the cell's chromosomes in a carefully coordinated process. However, chromosome number varies dramatically between genomes, even on short evolutionary timescales. We sought to understand how the mitotic machinery senses and responds to karyotypic changes by using a set of budding yeast strains in which the native chromosomes have been successively fused. Using a combination of cell biological profiling, genetic engineering, and experimental evolution, we show that chromosome fusions are well tolerated up until a critical point. However, with fewer than five centromeres, outward forces in the metaphase spindle cannot be countered by kinetochore-microtubule attachments, triggering mitotic defects. Our findings demonstrate that spindle architecture is a constraining factor for karyotype evolution.

419 **Proteome-wide alanine scanning with mistranslating tRNAs to identify functionally important residues across the proteome** Matthew D Berg, Alexis Chang, Kyle Hess, Ricard A Rodriguez-Mias, Judit Villén Genome Sciences, University of Washington

While DNA sequencing has identified millions of natural genetic variants that alter protein sequence, determining the functional impact of these variants remains challenging. Traditional mutagenic approaches are not scalable for millions of variants and high-throughput approaches such as deep mutational scanning are limited to investigating one protein per experiment. Recently, our lab established Miro – a high-throughput proteomic technology to enable functional annotation of thousands of missense mutations across entire proteomes simultaneously. In this approach, stochastic errors in protein synthesis are induced to create amino acid substitutions throughout all expressed proteins within a cell. Biochemical selections are applied to the collection of protein variants that probe general protein properties like solubility, thermal stability, ligand binding, protein-protein interactions and posttranslational modifications. After selection, variants are quantified by mass spectrometry to determine the functional impact of each mutation on the measured property. In this work, we harness a collection of engineered alanine tRNAs that mis-incorporate alanine at almost all non-alanine codons to determine the impact of thousands of alanine substitutions proteome-wide in yeast. By applying thermal stability and solubility assays to the alanine substituted proteomes, we identify functionally important residues and protein regions. Our work represents the first proteome-wide alanine scan and provides insight into various aspects of protein biology including the structural and functional context underlying mutational sensitivity.

420 **Copy number variation alters local and global mutational tolerance** Pieter Spealman[1], Grace Avecilla[2], David Gresham[1] [1]Biology, New York University, [2]BioBus

Copy number variants (CNVs), duplications and deletions of genomic content, contribute to evolutionary adaptation, but can also confer deleterious effects and cause disease. Whereas the effects of amplifying individual genes or whole chromosomes (i.e., aneuploidy) have been studied extensively, much less is known about the genetic and functional effects of CNVs of differing sizes and structures. Here, we investigated Saccharomyces cerevisiae (yeast) strains that acquired adaptive CNVs of variable structures and copy numbers following experimental evolution in glutamine-limited chemostats. Although beneficial in the selective environment, CNVs result in decreased fitness compared with the euploid ancestor in rich media. We used

transposon mutagenesis to investigate mutational tolerance and genetic interactions in CNV strains. We find that CNVs confer novel mutational tolerance in amplified essential genes and novel genetic interactions. We validated a novel genetic interaction between CNVs and BMH1, which we identified in multiple strains. We analyzed global gene expression and found that transcriptional dosage compensation does not affect the majority of genes amplified by CNVs, although gene specific transcriptional dosage compensation does occur for approximately 12% of amplified genes. Furthermore, we find that CNV strains do not exhibit previously described transcriptional signatures of aneuploidy. Our study reveals the extent to which local and global mutational tolerance is modified by CNVs with implications for genome evolution and CNV associated diseases, such as cancer.

421 **Sc2.0: Design and build a synthetic eukaryotic genome in yeast from scratch** Yu Jeremy Zhao[1], Camila Coelho[1], Amanda Hughes[2], Luciana Lazar-Stefanita[1], Sandy Yang[1], Leslie Mitchell[1], Joel Bader[3], Giovanni Stracquadanio[4], Lars Steinmetz[2,5], Yizhi Cai[6], Jef D. Boeke[1] [1]Institute for Systems Genetics, NYU Langone Health, [2]European Molecular Biology Laboratory, [3]Department of Biomedical Engineering, Johns Hopkins University, [4]School of Biological Sciences, The University of Edinburgh, [5]Department of Genetics and Stanford Genome Technology Center, Stanford University, [6]Manchester Institute of Biotechnology, The University of Manchester

Rapid advances in DNA synthesis technology enable transitioning from genome reading and editing to genome writing. The synthetic yeast project (Sc2.0) is building a designed synthetic genome in yeast from scratch. We have accomplished a significant milestone with the complete assembly of all individual synthetic chromosomes and consolidated seven of them into one yeast cell through endoreduplication intercross. This strain, with its genome more than half synthetic, carried ~6000 designer modifications and still grows similarly to the wild type. Here, we describe the next-generation consolidation strategy called chromosome substitution to combine all remaining synthetic chromosomes to build the ultimate strain with an entirely artificial genome. Using an innovative CRISPR-based genome scanning method, we discovered an unexpected genetic interaction that links transcriptional regulation, inositol metabolism, and tRNA abundance.

Synthetic chromosomes in Sc2.0 carry thousands of loxPsym sites, which enables a genome-wide shuffling system with an inducible Cre recombinase, a means to rearrange the synteny of protein-coding genes on demand. We are pioneering this evolutionary platform to answer a fundamental question: What constitutes the minimal gene set for eukaryotic cell growth? In addition, synthetic genomes represent a revolutionary approach to metabolic engineering. Our engineered Sc2.0 strain already showed improved isobutanol production. This observation inspired us to consider constructing the second designer genome of the premiere industrial yeast, *Kluyveromyces marxianus* (Kmarx2.0). This initiative aims to address the global environmental challenge of biofuel production directly.

422 **Visual impairment cell non-autonomously dysregulates canonical integrated stress response brain-wide** Shashank Shekhar[1], Katherine J Wert[2], Helmut Krämer[3] [1]UT Southwestern Medical center, [2]Opthalmology, UT Southwestern Medical Center, [3]UT Southwestern Medical Center

Recent statistical findings have pointed to an aspect of blindness that has garnered little attention in the past: blindness is a significant risk factor for dementia, a devastating set of diseases impacting more than 3 million people in the U.S. each year [1]. While highly intriguing [2], this finding currently lacks any mechanistic foundation.

Starting with 6 different Drosophila models of blindness, we observed the cell non-autonomous induction of stress granules (SGs) deep in the brain, and their reversal upon restoration of vision. These SGs contain cytosolic condensates of p62, ATF4 and XRP1 and are marked by the presence of RNA-binding proteins Caprin and dFMR1. This cytosolic restraint of the ATF4 and XRP1 transcription factors dampens their activity during integrated stress response (ISR). Brain-wide propagation of ISR dysregulation depends on specific lamina neurons as their thermogenetic manipulation triggered the reversible formation of SGs deep in the brain. We also demonstrate the evolutionary conservation of blindness-induced proteostasis dysregulation in mammalian brains. In the thalamus and hippocampus of congenitally blind vsx2 mice, or degenerative blind RhoP23H retinitis pigmentosa mice, we observed increased cytosolic condensates of p62 and ATF4.

In summary, we have discovered a functional interplay between the ISR and the formation of SGs in the brain as a strategy to prevent cell death under chronic stress. Our data reveal a previously unknown mechanism cell non-autonomously regulating protein quality control and provide mechanistic insight into blindness-induced proteostasis dysregulation deep in the brain. Malfunctioning of the proteostasis network is associated with a wide range of human diseases, especially neurodegeneration including dementia.

References:

1. J. R. Ehrlich, J. Goldstein, B. K. Swenor, H. Whitson, K. M. Langa, P. Veliz, (2022) Addition of Vision Impairment to a Life-Course Model of Potentially Modifiable Dementia Risk Factors in the US. JAMA Neurol 79, 623-626.

2. P. Span, The New York Times. (New York, NY, July 3rd, 2022).https://www.nytimes.com/2022/07/03/health/dementia-treatment-behavior-eye-care.html

423 **Splice variants of the actin nucleator Arp2 functionally diverge in fertility and development** Jordan Powell, Courtney M Schroeder Pharmacology, UT Southwestern Medical Center

The Arp2/3 complex is highly conserved throughout eukaryotic evolution and plays an essential role in branched actin nucleation. Interestingly, most eukaryotes encode two splice variants of Arp2 that differ merely by five amino acids in the unstructured D-loop, which is critical for Arp2/3 activation. Although the length of the alternative exon is largely conserved from flies to humans, the sequence is not. We investigated whether the *Drosophila* Arp2 splice variants functionally diverge by replacing *Arp2* in *D. melanogaster* with the coding sequence of either the short ('Arp2S') or long ('Arp2L') variant, each under the control of the Arp2 promoter. We expected minimal phenotypic differences because the splice variants are 99% identical in sequence. While both Arp2S and Arp2L fully rescue the *Arp2*-knockout lethality phenotype, we surprisingly found that *Arp2L*-expressing flies develop significantly slower than *Arp2S*-expressing flies. Furthermore, the *Arp2L*-expressing females are subfertile with a striking reduction in egg production, and males exhibit defects in actin cones, which are testis structures that separate syncytial sperm. Because the alternative exon in Arp2L differs in sequence between vertebrates and insects, we tested whether sequence or length contributes to these phenotypes by substituting the exon with five glycine residues. Surprisingly, the mutant exhibited normal development and fertility, suggesting that the alternative exon's sequence, not length, is disadvantageous in specific tissues, including the germline. We compared the motility of Arp2S- versus Arp2L-generated actin structures by live imaging the movement of cones, which are powered by actin polymerization. While Arp2S-generated cones move consistently down sperm tails at a speed similar to WT cones, Arp2L-generated cones move inconsistently, varying in speed within and across testes, suggesting the five additional residues alter actin polymerization kinetics. Overall, our data reveal that Arp2 splice variants are functionally nonequivalent due to the alternative exon's sequence, and we hypothesize that evolution shaped the Arp2L D-loop to tune actin polymerization kinetics for tissue- and species-specific roles.

424 **The splicing regulator Acn moonlights as a cytosolic activator of autophagy in a Tor-independent pathway to suppress neurodegeneration** Nilay Nandi[1], Leonard Yoon[2], Jeffery Kelly[2], Helmut Kramer[3] [1]Neuroscience, Univ of TX Southwestern Medical Center, [2]Scripps Research, [3]Neuroscience, UNIV OF TEXAS SOUTHWESTERN MEDICAL CENTER

To remove cellular debris and maintain homeostasis, neurons employ basal autophagy, a process that is positively regulated by Acinus (Acn) protein [1]. In Drosophila and mammalian cells, Acn is a primarily nuclear protein. The ASAP complex, formed by Acn together with Sap18 and RNPS1, binds to spliceosomes and regulates a subset of alternative splicing [2, 3], suggesting that Acn may stimulate autophagy through its function in alternative splicing. To test this, we generated the Acn^{ASAP} mutant that interrupts its binding interface with Sap18 and RNPS1 and, as predicted, interferes with ASAP-mediated alternative splicing. However, the Acn^{ASAP} protein is primarily cytosolic and enhances autophagy indicating that this mutant separates the regulatory functions of Acn in splicing and autophagy. Further support for a splicing-independent, non-nuclear role of Acn came from expression of a membrane-enriched myristoylated Acn that robustly enhanced autophagy. Moreover, an Acn^{NES} mutant that enhanced nuclear occupancy reduced autophagy, whereas an Acn^{NLS} mutant with reduced nuclear occupancy enhanced autophagy. These results are consistent with a model that, despite its primarily nuclear localization, Acn must exit the nucleus to induce autophagy. Because wild-type Acn is degraded in the cytoplasm [1], we reasoned that modifications that stabilize Acn would promote its function in autophagy. Acn stability is enhanced by phosphorylation of a highly conserved $Serine^{437}$, which is regulated by the balance of the activities of Cdk5-p35 kinase complex and the metal-dependent Nil phosphatase. Consistent with the importance of this modification, the phosphomimetic Acn^{S437D} mutation partially suppressed neurodegeneration and extends life expectancy compared to wild-type flies. Moreover, a drug obtained from an unbiased screen for mTor-independent autophagy activators can trigger Acn-S437 phosphorylation, enhance neuronal autophagy and suppress neurodegeneration in Drosophila models of Alzheimer's disease.

1. Nandi, N., L.K. Tyra, D. Stenesen and H. Krämer, J Cell Biol, 2014. 207: 253-68.

2. Malone, C.D., et al., Genes Dev, 2014. 28: 1786-99.

3. Hayashi, R., D. Handler, D. Ish-Horowicz and J. Brennecke, Genes Dev, 2014. 28: 1772-85.

425 **Calcium imbalance underlies PS-deficiency induced mitochondrial damage** Yifan Zhou[1], Xiao Yang[2], Xun Huang[1] [1]Institute of Genetics and Developmental Biology, Chinese Academy of Science, [2]Shandong First Medical University

Orchestrated phospholipid composition is essential for cellular homeostasis. It is well known that phosphatidylserine (PS) is an important component of cellular membrane. Beyond its structural function, comparatively little is known about the regulatory function of PS. Here we uncovered a conserved, robust link between PS metabolism and Ca^{2+} homeostasis. In *Drosophila*, we identified Ca^{2+} signaling as a critical player downstream of PS-dependent mitochondrial integrity. We show that PS deficiency impairs mitochondrial function through triggering mitochondrial Ca^{2+} overload. The inositol trisphosphate receptor (IP3R) channel is involved in this process, forming clusters in response to PS deficiency. Mechanistically, reduced PS biosynthesis reshaped PE species profile and inhibited the activation of SREBP signaling. Correcting the lipid composition suppressed mitochondrial defects and formation of IP3R clusters. In mammalian cells, IP3R-dependent Ca^{2+} signals were sensitized by disease-associated status of PS synthesis: in the context of PS deficiency, IP3R activation elicited long-lasting Ca^{2+} spikes, with increased frequency and reduced duration of peaks as compared to the wild-type cells. While in the Lenz-Majewski syndrome (LMS)-causative mutant cells, where PS is overproduced, IP3R activation induced higher amplitude of Ca^{2+} transient than that in the wild-type cells. Our data suggest that balanced Ca^{2+} signal allows cells combat against disorders induced by aberrant PS metabolism.

426 **Dual role of PP2A in Hippo signalling and growth regulation** Aashika Sekar[1], Elodie Sins[2], Alberto Rizzo[2], Paulo Baptista-Ribeiro[2] [1]Barts Cancer Institute, Queen Mary University London, [2]Barts Cancer Institute

The Hippo pathway integrates multiple environmental signals to regulate tissue growth and homeostasis. Previously, we demonstrated that the apical-basal polarity protein Crumbs inhibits Hippo signalling by promoting CK1α kinase-dependent phosphorylation and degradation of upstream Hippo activator, Expanded. Here, using *in vitro* biochemical approaches in *Drosophila* S2 cells and *in vivo* approaches in the larval wing disc, we provide evidence that PP2A activates Hippo signalling by (1) antagonising Crumbs-mediated phosphorylation and degradation of Expanded and (2) stabilising basal Expanded levels in the absence of Crumbs stimulus. This occurs independently of the PP2A-containing STRIPAK complex, which inhibits the Hippo pathway by dephosphorylating Hippo. Importantly, we also show that distinct PP2A complexes are involved in the regulation of basal and Crumbs-mediated stabilisation of Expanded, thereby suggesting that Crumbs directs the interaction between PP2A and Expanded. Furthermore, we observed similar PP2A-mediated regulation of FRMD-6, the human orthologue of Expanded, in *Drosophila* S2 cells. Overall, our work characterises a novel Hippo-activating role for PP2A in the stabilisation of Expanded and provides insight into how PP2A tightly controls Hippo pathway in response to polarity stimuli.

427 **New A.I. behavioral annotation system MAFDA reveals that Fruitless robustly controls interaction social behaviors by guiding lipid metabolism.** Jie Sun[1], Wen-Kan Liu[1], Calder Ellsworth[1], Qian Sun[2], Yu-Feng Pan[3], YICHUN HUANG[1], Wu-Min Deng[1] [1]Tulane University School of Medicine, [2]Louisiana State University, [3]Southeast University

Pheromones are chemical signals used for communication between members of the same species. To increase fitness, animals use both internal (pheromone perception) and external states (pheromone production) to coordinate multiple physiological behaviors. In *Drosophila*, the male-specific isoform of Fruitless (Fru), FruM, is known as a master neuro-regulator of innate courtship behavior to control perception of sex pheromones in sensory neurons. Here we show that the non-sex specific Fru isoform (FruCOM) is necessary for pheromone biosynthesis in hepatocyte-like oenocytes for sexual attraction. Loss of FruCOM in oenocytes resulted in adults with reduced levels of the cuticular hydrocarbons (CHCs), including sex pheromones, and show altered sexual attraction and reduced cuticular hydrophobicity. We further identify evolutionarily conserved *Hepatocyte nuclear factor 4 (Hnf4)* as a key target of FruCOM in directing fatty acid conversion to hydrocarbons in adult oenocytes. *fru*- and *Hnf4*-depletion disrupts lipid homeostasis, resulting in a novel sex-dimorphic CHC profile, which differs from *doublesex*- and *transformer*-dependent sexual dimorphism of the CHC profile. Thus, Fru couples pheromone perception and production in separate organs for precise coordination of chemosensory communication that ensures efficient mating behavior. In addition, we developed a novel machine-learning-based automatic fly-behavioral detection and annotation (MAFDA) system, which allows us to visualize behavioral phenotypes under a temporal and spatial overlay, and to quantifiably compare behavioral index across different genetic backgrounds. This artificial intelligence system allows us to annotate social behaviors more accurately and digitize them, clustering them for a better comprehensive assessment of health indices. This system has been used in the behavioral assessment of liver injury models and cancer models, revealing the close relationship between disease ecology and interactive social behaviors.

428 **Glut1 functions in insulin-producing neurons to regulate organismal lipid storage in *Drosophila*** Matthew Kauffman, Justin DiAngelo Penn State Berks

Obesity continues to prevail as one of the largest health problems in the world. Obesity arises from excess storage of triglycerides (TAG); however, the full complement of genes important for regulating TAG storage is not known. The *Glut1* gene encodes a *Drosophila* glucose transporter that has been identified as a potential obesity gene through a buoyancy screen. However, the mechanism by which Glut1 acts in different tissues to affect the metabolism and storage of carbohydrates

and lipids is not fully understood. In this study, we characterized the role of Glut1 specifically in the fly brain. We decreased neuronal Glut1 levels by inducing RNAi in all neurons using *nSyb-Gal4* and measured the levels of TAG in adult flies. *Glut1-RNAi* flies had a decrease in TAG, and this was not due to changes in food consumption. A group of hormones that regulate metabolism and are expressed in the fly brain are *Drosophila* insulin-like peptides (Ilps) 2, 3, and 5. Since the secretion and expression of certain Ilps are also regulated by sugar, we hypothesized that decreasing *Glut1* in all neurons would result in a defect in the secretion and/or expression of these Ilps. Interestingly, we observed a decrease in the expression of *Ilp3* in *Glut1-RNAi* flies, suggesting that Glut1 functions in insulin-producing neurons (IPCs) to regulate organismal TAG. To test this hypothesis, we induced *Glut1-RNAi* specifically in the IPCs and measured TAG. We saw a decrease in the organismal storage of TAG in these flies as well as a decrease in the expression of *Ilp3*, indicating Glut1 functions in IPCs to regulate insulin-like peptide expression and whole-organism nutrient storage. If this decrease in *Ilp3* expression results in decreased TAG storage, we predict that genetically decreasing *Ilp3* would result in this same phenotype. To test this hypothesis, we measured TAG storage in *Ilp3* mutants and observed a decreased lipid phenotype, suggesting that the decreased *Ilp3* expression observed in *Glut1-RNAi* flies is responsible for the decreased TAG phenotype we observed in these flies. Together, these data suggest that Glut1 functions as a nutrient sensor in the insulin-producing cells, controlling TAG storage and regulating organismal energy homeostasis.

429 **Polarized recycling of cell-cell adhesion proteins facilitates scarless wound healing** Sofia Mendez Lopez, Kate MacQuarrie, Katheryn Rothenberg, Rodrigo Fernandez-Gonzalez University of Toronto

Following myocardial infarction, wound healing causes the formation of scar tissue, which limits cardiac output and can lead to heart failure. In contrast, embryos have a striking ability to repair wounds rapidly, with no inflammation or scarring. We are investigating the mechanisms of scarless wound repair in the Drosophila embryo, which is amenable to live imaging, as well as genetic, pharmacological and biophysical manipulations. In embryos, wound repair is driven by the collective movement of the cells around the wound to draw the wound closed. Upon wounding, the cells adjacent to the wound polarize the cytoskeletal protein actin and the molecular motor non-muscle myosin II, forming a supracellular cable at the wound edge that creates tension and coordinates cell movements. In parallel, adherens junction proteins, including E-cadherin, are depleted from former bicellular junctions (BCJs) at the wound edge and accumulate at former tricellular junctions (TCJs) around the wound. The reorganization of cell-cell adhesions is necessary for rapid wound healing. E-cadherin is removed from BCJs at the wound edge via polarized endocytosis, but the mechanisms by which E-cadherin accumulates at TCJs around the wound are unclear. To examine whether protein recycling contributes to E-cadherin reorganization during wound closure, we manipulated the activity of the small GTPase Rab11, which marks vesicles for slow recycling. Reducing Rab11 activity by overexpressing a dominant-negative form (Rab11DN), slowed down wound closure by 29%. While myosin polarization occurred normally in Rab11DN embryos, the accumulation of E-cadherin at TCJs decreased by 33%. Together, these results indicate that Rab11 is partially responsible for the remodeling of cell-cell adhesions during wound repair, and that cell-cell adhesion rearrangements control the rate of wound healing independent of cytoskeletal polarity. We are currently investigating the contribution of other protein trafficking pathways to embryonic wound healing, including rapid recycling (Rab8) and exocytosis (Sec5). Together, our results will pave the way for understanding the mechanisms of scarless wound repair, and may reveal novel therapeutic targets to prevent scarring and reduce the likelihood of heart failure following myocardial infarction.

430 **Genetic Quality Control for Mouse-Based Research: The Experience of the MMRRC** Matthew W Blanchard[1,2], Jennifer Brennan[1], James Amos-Landgraf[3], Renee Araiza[4,5], Laura Reinholdt[6], Brandon Willis[4,5], Dominic Ciavatta[1,2], Leonard W McMillan[7,8], John S Sigmon[7,8], Chidima Ahulamibe[9], Sam Ardery[2], Timothy A Bell[2], Pablo Hock[2], Caroline E.Y. Murphy[9], Joyce Woo[9], Fernando Pardo Manuel de Villena[2,8,10] [1]Genetics, Mutant Mouse Resource and Research Centers, University of North Carolina at Chapel Hill, [2]Genetics, University of North Carolina at Chapel Hill, [3]Mutant Mouse Resource and Research Centers, University of Missouri, [4]Mutant Mouse Resource and Research Centers, University of California at Davis, [5]Mouse Biology Program, University of California at Davis, [6]Mutant Mouse Resource and Research Centers, The Jackson Laboratory, [7]Computer Science, University of North Carolina at Chapel Hill, [8]Mutant Mouse Resource and Research Centers, University of North Carolina at Chapel Hill, [9]University of North Carolina at Chapel Hill, [10]Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill

The Mutant Mouse Resource and Research Centers (MMRRC) is the premier repository and distributor of spontaneous and induced mutant mice and cell lines for the US National Institutes of Health. As part of our commitment to optimize the rigor and reproducibility of biomedical research using mouse models, the MMRRC has implemented standardized Genetic Quality Control (GQC) for the models it distributes. This GQC process is used to update, validate, supplement, and correct information provided by strain donors, in a consistent, objective, and genome-wide manner. For each strain, the MMRRC Genetics Committee leverages MiniMUGA genotyping data from two independent mice from the MMRRC colony to update the MMRRC Strain Detail Sheet (SDS) in a section named MMRRC Genetic QC. This new section provides a detailed summary of the genome-wide makeup of the strain (including the Y Chromosome, the mitochondria, and the vendor origin for popular

substrains), the level of inbreeding and congenicity, and constructs present. We present real-world examples of curated MMRRC strains, detail the resulting SDS changes, and show how GQC provides more accurate and objective information for users. We will discuss how GQC may provide actionable recommendations that will improve experimental design and increase rigor and reproducibility. Currently we have genotyped 245 strains and completed the MMRRC GQC strain review in 41. At least a quarter of strains reviewed require an update or correction, involving strain name changes, overestimated level of congenicity and inbreeding, Y Chromosome origin discordant with naming, complex genetics, and the presence of unexpected constructs. We provide a summary of MMRRC strains with significant updates or corrections. For a subset of these strains, we will propose specific actions to address the impact(s) of the GQC findings including changing the suggested control mice, breeding out undesired constructs or Y Chromosomes, and adjusting experimental sample size to account for genetic heterogeneity in the line. Finally, we introduce two MMRRC-produced video tutorials, one designed to familiarize non-expert users with the MiniMUGA genotyping platform and a second explaining MMRRC GQC and how to interpret the relevant sections of the SDS.

431 **What's new in Mouse Genome Informatics (MGI)?** Carol J Bult, Martin Ringwald, Cynthia Smith, Richard Baldarelli, Joel Richardson The Jackson Laboratory

The Mouse Genome Informatics (MGI) consortium maintains multiple integrated community knowledgebases that are united by a common mission to facilitate the use of the laboratory mouse as an experimental model for understanding the genetic and genomic basis of human health and disease. MGI is the authoritative source for key data types and annotations including: official nomenclature for mouse genes, alleles, and strains; phenotype annotations; functional annotations, developmental gene expression; and mouse models of human disease.

Content and user interfaces are constantly evolving at MGI in response to community needs and to the rapidly changing data landscape. Likewise, the infrastructure that supports expert curation of information from the scientific literature is changing with advances in machine learning and artificial intelligence. This presentation will highlight some of the latest enhancements to MGI and plans for the near future.

Among the new features to be highlighted are:

- New methods and interfaces for mapping mouse phenotype terms to equivalent human phenotype ontology terms
- Visualization and interaction with genome annotations from multiple inbred mouse strains and other species (including human)
- Enhanced annotation of mouse genome variation
- Enhanced representation of regulatory genome features and alleles in the mouse genome
- Enhanced representation of the pseudoautosomal region (PAR) in the mouse
- Implementation of machine learning algorithms to improve efficiency of literature curation

MGI is supported by NIH HG000330, HD062499, CA089713, HG012212 432 ***The panoramic view of organismal aging at single-cell resolution*** Zehao Zhang, Chloe Schaefer, Weirong Jiang, Wei Zhou, Junyue Cao The Rockefeller University

Aging is a multifaceted biological phenomenon characterized by intricate changes in diverse cell populations throughout the entire organism. The identification and characterization of these cell types, especially those undergoing substantial alterations during the aging process, represent a crucial step in developing targeted interventions to mitigate or reverse aging-related degeneration. Despite remarkable advancements in single-cell genomics, existing methodologies face challenges in capturing rare yet pivotal cell types involved in aging at the organismal level. In this study, we developed a high-throughput genomic analysis platform that is suitable for organismal-scale single-cell transcriptome profiling. With this platform, in one study, we profiled more than 20 million high-quality single-cell transcriptomes across 13 distinct organs from a sex-balanced mice cohort, yielding the most extensive catalog of aging-associated cell types (i.e., 230 distinct cell populations that undergo significant expansion or depletion), each characterized by its unique gene expression profiles and cell state dynamics. Notably, the scalability of our platform has effectively mitigated batch effects that typically arise when integrating various single-cell datasets in conventional consortium-level studies. This panoramic assessment of aging has revealed four temporally structured, dynamic aging waves that are organ-specific and lineage-specific. Additionally, we have explored the aging-related alterations in immune cell populations and their interplay with other cell types. The experimental and computational resources outlined here are expected to enhance our understanding of aging, fostering innovative methodologies to extend healthy

lifespans and address age-related diseases.

433 **Generation of knock-in rodent models using AAV-mediated DNA delivery with 2-cell embryo CRISPR-Cas9 electroporation** Daniel Davis, James McNew, Bhanu Telugu, Elizabeth Bryda University of Missouri

Complex animal models with targeted DNA insertions or substitutions (knock-ins) are essential for a variety of applications. In many cases these knock-ins are required to be large in size, which has been shown to reduce gene editing efficiency and limit capabilities of successfully creating the desired model. Numerous technical refinements have been developed to increase CRISPR-Cas mediated genome editing pertaining to creating targeted knock-ins via the homology-directed repair (HDR) pathway. In this study we have merged four promising approaches to come up with a highly effective pipeline to generate knock-in mouse and rat models. The four combined methods include: AAV-mediated DNA delivery, single-stranded DNA donor templates, 2-cell embryo modification, and CRISPR-Cas ribonucleoprotein (RNP) electroporation. Using this optimized approach, we have successfully produced targeted knock-in mouse and rat models carrying insertion of DNA sequences up to 3.0 kb in size with efficiencies of over 90%. Our modified AAV-mediated DNA delivery with 2-cell embryo CRISPR-Cas9 RNP electroporation technique has proven to be highly effective for generating both knock-in mouse and knock-in rat models.

434 **Algorithmic prioritization of genetic background for improved rare disease model generation** Robyn L Ball[1], Gaurab Mukherjee[1], Alexander Berger[1], Sara Davis[2], John Bluis[2], Robert Duggan[1], Mathew Gerring[1], Tim Holland[2], Beena M Kadakkuzha[1], Guy Karlebach[2], Peter Robinson[2], Elissa J Chesler[1] [1]The Jackson Laboratory, [2]JAX Genomic Medicine

Mouse models for rare disease research are often generated by modeling human genetic variants through genome editing of a small handful of widely used background strains, often chosen without insight into the susceptibility of the strain to the disease in question. Even single gene perturbations (i.e., rare, Mendelian disease variants) function in the context of biological networks. Some networks are highly robust and are able to buffer the effect of deleterious alleles readily. Others are molecular networks and are vulnerable to the effects of allelic variation. Some mouse strains have more deleterious allele in these networks than others, and we posit that these cumulative effects influence vulnerability to rare variants, resulting in variable penetrance and impact of disease mutations. Such strains may show mild patterns of network level dysfunction. These strains are detectable through the manifestation of similarity to disease associated phenotypic or transcriptional states. By imputing these states from molecular and physiological phenomes in all extant mouse resources, we can identify those strains that most resemble people with disease vulnerability and thus identify the ideal background strains in which to model disease. We present the application of our software pipeline for prioritization of these strains. Several examples are presented in rare and common disease, along with considerations for background strain selection from the prioritized results. The JAX Center for Precision Genetics Bioinformatics Core Strain Recommender uses molecular and phenotypic signals of disease to recommend mouse backgrounds likely to be susceptible (and resistant) to the disease and treatment. This work is supported by NIH JCPG grant number, NIH DA028420 and by The Jackson Laboratory, The Cube Initiative Program Fund.

435 **The NIH Comparative Genomics Resource (CGR)** Terence D Murphy, Nuala O›Leary, Sanjida Rangwala, Francoise Thibaud-Nissen, Tom Madden, Aron Marchler-Bauer, Valerie Schneider NCBI/NLM, NIH

Comparative genomic analyses are a powerful tool for scientific discovery. CGR is an NIH-funded, multi-year NLM project to establish an ecosystem to facilitate reliable comparative genomics analyses for all eukaryotic organisms in collaboration with the genomics community. The project's vision is to maximize the biomedical impact of eukaryotic research organisms and their genomic data resources to meet emerging research needs for human health. To achieve this, NCBI will provide high-value data and assorted tools compatible with community-provided resources. This presentation will give an overview of CGR resources of interest to the TAGC community, including genome contamination screening and annotation tools, new methods to access genome and gene sequence and metadata, Comparative Genome Viewer (CGV) for pairwise analysis of genomes, and improvements to BLAST and domain analysis to aid in exploring protein function and conservation across the tree of life.

436T **Identification and verification of proximity interactors of the polycystin complex in cilia and extracellular vesicles** Elizabeth desRanleau[1], Inna Nikonorova[2], Katherine Jacobs[2], Joshua Saul[2], Jonathon Walsh[2], Juan Wang[2], Maureen Barr[2] [1]Genetics, Rutgers University, [2]Rutgers University

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a devastating genetic disorder characterized by fluid-filled cysts that

accumulate in the kidneys, leading to renal failure. ADPKD is caused by mutations in the polycystin complex, encoded by PKD1 and PKD2. The polycystins localize in cilia of kidney cells and human urinary extracellular vesicles (EVs), but their function in EVs remains unexplored.

In C. elegans, like in humans, polycystins may act in cilia and EVs. In the worm, the polycystins are male-specific and are necessary for mate sensing by males. The polycystin complex, LOV-1 and PKD-2, is expressed in the tail ray neurons (RnBs) and cephalic male sensory neurons (CEMs). When either polycystin is mutated, male worms are mating deficient. With whom the polycystins interact in cilia and EVs is not known. Here, we used proximity labeling to identify interactors of the polycystin complex that colocalize into ciliary EVs. The objective of this work is to validate candidate cargo of polycystin-carrying EVs in *C. elegans* with endogenous genome-engineered fluorescent reporters.

Here, we focused on two novel PKD-2-proximity interactors: the polycystin-associated transmembrane lectins PAML-1 and PAML-2. When tagged with fluorescent reporters, we found that these proteins colocalize with PKD-2 in cilia and EVs. PAML-1 and PAML-2 are male-specific and are expressed in non-overlapping *pkd-2* expressing neurons. In L4 larval males, *paml-2* is expressed in all ray neurons then declines in ventral neurons during sexual maturation. In contrast*, paml-1* expression is limited to the ventral ray neurons. We discovered that although PAML-2 is initially present in all *pkd-2* expressing neurons, PAML-2 localizes to the cilia of dorsal rays and CEMs, whereas PAML-1 localizes to the cilia of ventral rays. When *lov-1* is mutated, neither PAML-1 nor PAML-2 are delivered to the cilium from the cell body, which suggests that LOV-1 is required for the transportation of these proteins to the ciliary tip. Our data shows that PAML-1 and PAML-2 are markers of dorsal and ventral EVs, where association with the polycystin complex might act as the factor that determines dorsal versus ventral transport of these lectins to the ciliary tip.

437T **Getting stressed out – iron induced stress response in *C. elegans*** Anna Gremme[1], Zainab Al-Timimi Safa Flaih[1], Bernhard Michalke[2], Julia Bornhorst[1] [1]Food Chemistry with focus on Toxicology, University of Wuppertal, [2]Helmholtz Munich

As an essential trace element, the general population mainly takes up iron (Fe) in form of various Fe species through food, food supplements and drinking water. The redox capacity between the oxidation states Fe^{2+} and Fe^{3+} turns Fe into an important key player in many biological processes. In addition, the adequate formation of reactive oxygen and nitrogen species (RONS), e.g. by Fe via Fenton reaction, is necessary for different signaling pathway cascades. However, an enormous Fe-induced RONS formation upon Fe overdosage lead to oxidative stress and is supposed to be a risk factor of various diseases.

Since little is known about the consequences and underlying mechanisms of overdosing different Fe species, we treated the nematode *C. elegans* with iron (III) ammonium citrate (FAC), iron (II) gluconate (FeGlu), and iron (II) chloride ($FeCl_2$) for 5 and 24 hours. We quantified the Fe by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and observed overdosing in the worm by all Fe species. Increased superoxide dismutase (SOD) activity and MitoTracker™ red CM-H_2Xros fluorescence indicate the formation of RONS by all Fe species, with SOD activity altering in a species-specific manner. While the Fe-species have no effect on the mRNA expression of the genes *daf-16* (human *FOXO*) and *skn1* (human *NRF2*), measured *via* Taqman qPCR, a species-specific increased translocation of DAF-16 in the nucleus can be observed in a DAF-16:GFP *C. elegans* mutant. Since glutathione is an important molecule of the antioxidant system, we measured reduced (GSH) and oxidized (GSSG) glutathione *via* liquid chromatography–tandem mass spectrometry (LC-MS/MS). While no changes could be observed after FeGlu and $FeCl_2$ treatment, FAC shows increased GSH levels. Measurements of mRNA expression of *gcs-1* (human *GCLC*) and *gst-4* (human *HPGDS*) *via* Taqman qPCR indicate reduced gene expression by treatment with all Fe species after 24 hours. In this context we were interested in consequences of Fe overdosing in a worm model with depleted GSH. GSH depletion was achieved by diethyl maleate (DEM) after FAC incubation and verified *via* LC-MS/MS analysis.

In this study, we can show that the stress response after Fe overdosage in *C. elegans* is species-specific in some oxidative stress endpoints. This is an important finding to better assess mechanistic effects in a species-specific way. Further consequences and underlying mechanisms of Fe overdosage, also in combination with GSH depletion, are the subject of current studies.

438T **Investigating the role of a conserved 14-3-3 protein, FTT-2, in contractility of smooth muscles** Mustafi Raisa Amin Biology, Northeastern University

Cell contractility is vital for the maintenance and regulation of several biological systems, including the circulatory, respiratory, and digestive systems, etc. To study cell contraction, we use the *C. elegans* spermatheca, a contractile tube composed of smooth muscle-like cells, that stores sperm and is the site of oocyte fertilization. Regulated spermathecal contraction is needed to push the embryo into the uterus. Spermathecal cell contraction is regulated by actin and myosin, and is activated, in part, by the phospholipase, PLC-1. Previous studies have shown that the heterotrimeric G-protein alpha subunit, GSA-1/Ga_s, and protein kinase KIN-1/PKA-C are involved in ovulation and Ca^{2+} release. However, the upstream regulators of these pathways are still unknown. The spermatheca is not under neuronal control. So, how do the spermathecal cells know when to

contract? In order to identify novel regulators of spermathecal contractility, we performed a candidate RNAi screen to look for genes that caused an increase in the number of occupied spermathecae. One of the candidate genes, *ftt-2*, is one of the two putative 14-3-3 encoding genes in C. elegans. The 14-3-3 proteins have been implicated in a growing number of cell biology processes, however not much is known about their involvement in regulation of cellular contraction. Hence, study of the FTT-2 gene may identify a novel function of the 14-3-3 proteins in general.

439T **The acyl-CoA dehydrogenase ACDH-11 is required for *C. elegans* lysosomes-related organelle biogenesis** Madeline Daniel, Frances Parrott, David Nhek, Frances Courtemanche, Greg Hermann Lewis & Clark College

Gut granules are lysosome-related organelles (LROs) that coexist with conventional lysosomes within *C. elegans* intestinal cells. Gut granules contain conspicuous crystalline and autofluorescent material and function in lipid metabolism, cell signaling, and heme and micronutrient storage. The formation of gut granules requires evolutionarily conserved proteins that broadly function in LRO biogenesis. We screened *C. elegans* Million Mutation Project strains for mutants with altered LRO dynamics and identified a role for the ACDH-11 acyl-CoA dehydrogenase in gut granule formation and morphology. We show that *acdh-11(-)* mutant embryos exhibit enlarged gut granules and defects in the steady-state localization of the gut granule protein LMP-1. The morphology of conventional degradative lysosomes, lipid droplets, and yolk granules are unaltered in *acdh-11(-)* mutants. Gut granule numbers are unchanged in *acdh-11(-)*, suggesting that ACDH-11 activity impacts trafficking to/from gut granules rather than organelle fusion. ACDH-11 has been shown to inhibit the expression of the lipid desaturase FAT-7 in adult intestinal cells. We find that *acdh-11* is likely expressed embryonically and that FAT-7 becomes expressed in embryonic intestinal cells in *acdh-11(-)* mutants. The removal of *fat-7* activity in *acdh-11(-)* mutants reduces gut granule size and the overexpression of FAT-7 in *acdh-11(-)* results in gut granule enlargement, showing that FAT-7 activity promotes the expansion of gut granules in *acdh-11(-)*. Ectopic expression of FAT-7 in *acdh-11(+)* did not increase gut granule size, indicating that other processes altered in *acdh-11(-)* synergize with FAT-7 activity to promote gut granule enlargement. FAT-7 promotes the generation of unsaturated fatty acids to increase membrane fluidity. Consistent with increased membrane fluidity promoting gut granule enlargement, gut granules in *acdh-11(-)* mutants are significantly smaller at 15C compared to 22C. We will present work that investigates how ACDH-11 functions to regulate gut granule biogenesis.

440T **Enzyme kinetic characterization of wild-type *C. elegans* IDH-1 and the G98N & R133H mutants** Melissa A. Bouchard, Anne McAllister, William Wolfe, Katherine M. Walstrom Div. Natural Sciences, New College of Florida

The *C. elegans* ortholog of human cytosolic isocitrate dehydrogenase (IDH1) is called IDH-1. Isocitrate is reversibly converted to α-ketoglutarate (αKG) by both enzymes. Because some mutations linked to human malignancies can decrease the normal activity of IDH-1 and introduce a neomorphic enzyme activity that converts αKG to 2-hydroxyglutarate (2HG), this enzyme is of particular interest for cancer and genetics research. This experiment began with the overexpression and purification of wild-type IDH-1 as well as two mutants, G98N and R133H, which correspond to the human G97N and R132H mutations, respectively, to investigate whether IDH-1 may be a trustworthy model system to investigate the cellular abnormalities caused by IDH-1 mutations. During our initial characterization of wild-type IDH-1 using spectroscopic assays to detect NADPH, we found that the enzyme had increased activity in the presence of Mn^{2+} compared to $Mg^{2+}$and a strong preference for $NADP^+$ compared to NAD^+. In comparison to the previously published K_M values for αKG with the human R132H mutant, the experimental K_M for αKG for the *C. elegans* R133H mutant was lower (14 ± 2 μM). The findings of other experiments demonstrated that, in the forward reaction, human wild-type IDH1 and its R132H mutant shared traits with wild-type IDH-1 and the R133H mutant respectively. However, the G98N mutant had a higher forward enzyme activity than the human G97N mutant. LC-MS was used to measure the amounts of 2HG and isocitrate (ICT) produced by the enzymes in the reverse reactions, and the evidence indicated that the R133H mutant produced a larger ratio of 2HG per reaction (80 ± 20 2HG/ICT) than the G98N (13 ± 1 2HG/ICT) and wild type (1.5 ± 0.3 2HG/ICT) enzymes. (The first three authors contributed equally to this research project.)

441T **Actomyosin fibers in the spermatheca are under tension** Seyedeh-Fereshteh Sadeghian-Kiadehi[1], Erin Cram[2] [1]Bioengineering, Northeastern University, [2]Biology, Northeastern University

The proper control of contractile and relaxation processes is crucial for the functionality of tubular structures within organisms. In the case of *C. elegans*, the spermatheca, the site of fertilization, is comprised of cells that exhibit properties similar to smooth muscle. As oocytes pass through the spermatheca, the process involves several cycles of stretching and contracting. Within the spermatheca, actin structures take on a characteristic form, with parallel, basal bundles resembling stress fibers aligned along the cellular long axis. In order to gain a more comprehensive insight into how mechanical forces are transmitted within the spermatheca, we measured the tension exerted on these fibers employing a strain sensor named STReTCh. The STReTCh system consists of two components: SpyTag and SpyCatcher. SpyTag is an epitope tag integrated into a mechanosensitive domain, while SpyCatcher serves as a fluorescently labeled binding partner. SpyCatcher can only interact

with SpyTag when the mechanosensitive domain is in an unfolded state. In the context of *C. elegans*, we customized this technique by integrating the sensor into mechanosensitive sections of DEB-1/vinculin and FLN-1/filamin. Our observations revealed that SpyCatcher and DEB-1/vinculin::SpyTag, as well as SpyCatcher and FLN-1/filamin::SpyTag, coincide in the spermatheca when animals are subjected to *mel-11/MYPT* RNAi treatment and *plc-1/phospholipase C* RNAi treatment, respectively. Furthermore, we assessed the tension on the fibers through laser ablation. In animals expressing ACT-1::GFP, we employed a SpectraPhysics Spirit laser operating at 1040 nm, with 400 fs pulses at a frequency of 1 kHz and approximately 500 mW power, to sever the fibers, allowing us to observe the rates of fiber retraction. Protein kinase A, which consists of the catalytic subunit KIN-1/PKA-C and the regulatory subunit KIN-2/PKA-R, plays a pivotal role in controlling calcium release and contractility within the *C. elegans* spermatheca. Initial findings indicate that KIN-1/PKA has an influence on the tension of the fibers. The combined results from these methods point towards the presence of tension in the actomyosin fibers within the spermatheca. Our upcoming plan involves mapping tension distribution throughout the spermatheca and elucidating the role of PKA in regulating contractility.

442T **Mechanisms of Lamp1 non-cell autonomous regulation of endo-lysosomal acidification** Jonathan Chandler Handy[1], Aliza Kass[1], Gustavo MacIntosh[2], Andreas Jenny[1] [1]Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]Biochemistry, Biophysics and Molecular Biology and Plant Sciences Institute, Iowa State University

Proteostasis is critical for the maintenance of cellular homeostasis especially during aging. Disruption of proteostasis results in the accumulation of toxic and insoluble protein aggregates associated with diseases like Alzheimer's Disease (AD) and Parkinson's Disease (PD). Lysosomes are the major degradative organelle in cells and are responsible for degrading macromolecules including proteins. The degradation activity of acid hydrolases within lysosomes is highest at pH 4.5. Alterations in lysosomal pH are frequently linked to lower degradative capacity and thus intrinsically to aberrant proteostasis and neurodegenerative diseases. Lysosomal membrane integrity is protected from acid hydrolases by lysosomal associated membrane proteins, the most abundant of which are LAMP1 and LAMP2. Mounting evidence suggests that LAMP proteins associate with other lysosomal membrane proteins to regulate lysosomal acidification, though the mechanisms by which this happens remain unclear. *Drosophila melanogaster Lamp1* encodes the LAMP1/2 homolog, and data from our lab suggest deletion of functional Lamp1 protein leads to an accumulation of acidic vesicles in the endo-lysosomal compartment of fat bodies (FBs; akin to liver and adipose tissue), termed excessive endo-lysosomal acidification (EELA). Intriguingly, when individual *Lamp1* mutant FB cells are induced in mosaics of an otherwise *Lamp1* heterozygous fly, EELA is not seen. This finding suggests that *Lamp1* is either required in a tissue other than FB to prevent EELA or that *Lamp1* expression in neighboring FB cells is sufficient to rescue EELA in FB cells lacking *Lamp1*. We thus have identified an unprecedented non-cell autonomous function of Lamp1 in regulation of endo-lysosomal pH. We will report on the characterization of the pH and endo-lysosomal identity of the acidic vesicles and on the identification of tissues in which *Lamp1* is required and/or sufficient to regulate endo-lysosomal pH in the FBs. To date, we have determined that expression of *Lamp1* in FBs and neurons of *Lamp1*-deficient larvae are sufficient to suppress EELA.

Our approach to address Lamp1 function will provide much needed insight into the regulation of endo-lysosomal acidification critical for proteostasis. It will therefore significantly advance our understanding of how dysfunction of the endo-lysosomal system underlies disease, ultimately leading to the development of novel treatment strategies to improve healthspan.

443T **The Role of the ER Integral Protein Jagunal in Regulating "Stemness" of Neuroblasts During Asymmetric Cell Division.** Judy Especial Abuel, Blake Riggs Biology, San Francisco State University

Cell division is a fundamental process in all living organism as it is essential in the development of all multicellular organisms. This development is accomplished through an unequal asymmetric partitioning of cellular components during cell division. However, the regulation and mechanism which allows for asymmetric cell division (ACD) to lead to a diverse generation of cells is poorly understood. A recent study shows that asymmetric partitioning of the endoplasmic reticulum (ER), an essential organelle involved in a host of cellular events, may be involved in the adoption of cell fate and cell diversity through a highly conserved ER transmembrane protein called, Jagunal (Jagn). Cell fate determination is normally controlled by cell fate determinants and transcription factors that drive the intrinsic and extrinsic signaling pathways that allow genes to be turned on and off. Our preliminary data illustrates that the presence of Jagn is crucial in maintaining cell diversity and cell fate determination. We believe that the disruption of ACD due to lack of Jagn leads to a direct consequence of changes in the fate of the two daughter cells. Previous studies showed that Jagn is involved in the proper partitioning of the ER in pro-neural cell populations and our preliminary data revealed an increase of the NB population in Jagn-deficient larval brains, suggesting that Jagn is involved in ACD and in the correct localization of cell fate determinants. To determine whether Jagn regulates proper NB development, I will examine programed NB quiescence during embryonic development in Jagn-deficient embryos to distinguish whether this event happens prior to cell fate selection. I will incorporate the thymidine analog 5-ethynyl-2-deoxyuridine (EdU) in late-stage embryos and then fix the tissue in order to observe whether Jagn-deficient embryos bypass

quiescent state and instead allow NBs to actively divide during embryonic-to-larval transition. I hypothesize that Jagn is involved in cell fate decisions during mitosis, establishing a differentiated cell population. This study will determine if an ER-specific protein has a role in cell fate selection and ACD. Furthermore, our findings will shed light on important questions related to cell specification and signaling transport during cellular organization and partitioning of cell fate determinants, polarity establishment, and regulation of the ACD.

444T **Non-canonical dFOXO regulation in Drosophila larval oenocytes under starvation** Peiduo Liu, Hua Bai Iowa State University

The Forkhead box O (FOXO) transcription factors are the key regulators of metabolic homeostasis and aging. It is well known that upon nutrient deprivation, insulin signaling decreases and FOXO is dephosphorylated and translocated into the nucleus to activate the transcription of a large number of metabolic genes, including those in lipid metabolism pathways. Unexpectedly, we find that Drosophila dFOXO protein is localized to the nucleus of larval oenocytes under normal fed conditions, while starvation reduces nucleus-localized dFOXO. The non-canonical response to starvation suggests a unique dFOXO regulation in Drosophila larval oenocytes, the specialized hepatocyte-like cells. Consistent with this notion, our genetic analysis shows that oenocyte-specific knockdown of Akt does not alter the nuclear shuttling of dFOXO under fed and starvation conditions. To further dissect the nutritional signals that regulate the non-canonical dFOXO activation in larvae oenocytes, we examine the effects of macronutrient refeeding on starvation-induced dFOXO nuclear depletion. Interestingly, refeeding of proteins, but not sugar, can attenuate the starvation-induced dFOXO nuclear depletion, which suggests that amino acid deficiency is a key underlying mechanism for non-canonical dFOXO regulation in larval oenocytes. Lastly, we screen a panel of amino acids and find that a deficiency of lysine and phenylalanine, but not branching amino acids, is required for starvation-induced dFOXO nuclear depletion. It is known that, unlike other larval tissues, Drosophila oenocytes accumulate fat upon starvation, similar to hepatic steatosis in the mammalian liver. Our findings indicate that Drosophila larval oenocytes may activate distinct metabolic programs via non-canonical dFOXO regulation to maintain metabolic homeostasis during nutrition deprivation.

445T **Rcp, a regulator of G-protein-coupled receptor signaling, controls the polarized deposition of basement membrane proteins in epithelial cells** Lindsey Price[1], Rebecca Brnot[1], Trent Davids[1], Alejandro Salas[1], Tracie Yiqing Kong[2], Trudi Schüpbach[2], Olivier Devergne[1] [1]Northern Illinois University, [2]Princeton University

Epithelia are organized in sheets of tightly adherent cells that are polarized along an apical-basal axis. Epithelial cells rely on their cellular organization for their structure and functions. The apical-basal polarity is characterized by the cytoplasmic and surface organization of individual cells in apical, basal, and lateral domains. The establishment and maintenance of these different domains rely on the transport and sorting of newly synthesized or recycled proteins to their correct locations. A key component of this organization is the basement membrane (BM), a specialized sheet within the extracellular matrix. The BM lies along the basal side of epithelial cells and is essential for the establishment and maintenance of epithelial cell structure and functions, tissue organization, and organ morphogenesis. The loss of integrity and misregulation of the BM leads to epithelial defects which can cause changes in signaling and cellular communication and has been associated with carcinomas and tumor metastasis. The BM components are produced within the epithelial cells and are secreted to the basal side through a dedicated pathway. Despite the BM's important roles in epithelial organization, the process of the secretion of BM proteins is poorly understood. To study BM protein deposition, we use the follicular epithelium (FE) of the *Drosophila* ovary as a model system. In a genetic screen looking for new genes involved in the proper placement of BM proteins, we identified a new gene, *Rcp* (Receptor component protein), which has been shown to be involved in G-protein signaling. Our data show that the loss of *Rcp* leads to an apical mislocalization of BM proteins without primarily affecting the general polarity, indicating that *Rcp* specifically controls the proper placement of BM proteins. Interestingly, *Rcp* is the first component of a signaling pathway implicated in BM polarity. In the FE, Rcp assumes cytoplasmic and nuclear localizations, this data paired with Rcp's protein structure suggests a role of Rcp in gene regulation. Finally, we also showed that Rcp genetically interacts with other known members of the pathway dedicated to the polarized deposition of BM proteins such as PIP2, and the GEF Stratum. Altogether, our data indicate a specific role for *Rcp* in epithelial organization by regulating the polarized deposition of BM proteins, a process that is critical for the establishment and maintenance of epithelial cell polarity.

446T **Characterizing the epithelial sheath as a barrier to professional phagocytes during apoptosis in the ovary** Max C Wertheimer, Alexandra Chasse, Kim McCall Biology, Boston University

In Drosophila, when cells die they are typically removed by phagocytic hemocytes. However, the ovaries inhibit hemocyte infiltration even when signals are released during cell death events. The signals released typically attract phagocytic cells to break down developing egg chambers and recycle nutrients but while hemocytes have been seen on the periphery, they do not enter the ovarioles. The epithelial sheath, which lines the surface of ovarioles, may be responsible for inhibiting hemocyte infiltration via septate junctions which control the diffusion of signals across tissues and may physically inhibit hemocytes

themselves. Eleven septate junction genes were identified as candidates and, using RNAi, knocked down to determine if they play a role in barring hemocyte entry into the epithelial sheath. The ovaries were dissected, stained and imaged with confocal microscopy to visualize any potential phenotypes. Findings show that many of the selected genes have adverse effects on fecundity and suggest the possibility of increased cell death in the ovaries. Some genes show severe phenotypes ranging from large gaps between eggs, tight bunching of the epithelial sheath, bunching of early stage eggs, and stretched stalk cells between eggs. These atypical phenotypes suggest excessive cell death, improper relaxation of the epithelial sheath, and abnormal cell signaling. Some of the knockdowns also affect hemocyte invasion into the ovariole. Discovering the gene(s) responsible for epithelial sheath permeability can be key for understanding the immune response in other immune privileged organs and potentially develop targets for therapy.

447T **Mutagenesis and characterization of the putative HAD-domain phosphatase CG11291 in *Drosophila melanogaster*** Veronica Gomes[1], Jennifer Kennell[2] [1]Vassar College, [2]Biology, Vassar College

Phosphoglycolate phosphatase (PGP) is an enzyme that plays an important role in DNA damage repair in bacterial and mammalian cells, is involved in producing glycerol in mice and worms, and breaks down toxic side-products of glycolysis in human and yeast cells. So far, PGP has only been studied in a few organisms, and very little is known about its function in Drosophila. Therefore, this study attempts to characterize the gene, *CG11291*, which is one of the predicted orthologs of *PGP* in *Drosophila melanogaster*. Studying this ortholog could tell us more about the function of the enzyme in fruit flies, and whether it has evolved to perform novel functions in this organism. *CG11291* is a small gene (~1.87kb) located on the positive strand of the second chromosome of *Drosophila melanogaster*. This gene is predicted to encode a member of the HAD-domain phosphatase family and has been found to be expressed during the larval, pupal, and adult stages of development, mostly in the testes of adults. To learn about the role of *CG11291* in the metabolism or development of *Drosophila melanogaster*, we have mutated the gene in this species. Transgenic flies were generated that expressed two guide RNAs targeting *CG11291* under the control of the UAS enhancer to induce CRISPR-Cas9-mediated mutations in *CG11291*. After conducting controlled crosses of transgenic flies, two mutant lines with unique frameshift mutations were selected to be phenotyped. Currently, the mutant lines are being tested for their sensitivities to ethylene glycol, salt, and oxidative stress, as well as fertility. Additionally, *CG11291* has been annotated in nine different species of *Drosophila* to learn more about the evolution of this gene by comparing the levels of similarity of its gene structure, protein sequence, and genomic neighborhoods between the various species.

448T **Regulation and function of GAGA factor mitotic retention in the early *Drosophila* embryo** Annemarie E Branks, Marissa Gaskill, Hope Hawthorn, Kerstin Hurd, Olivia Ward, Melissa Harrison University of Wisconsin-Madison

Development is driven by cell division and differentiation, which is controlled by gene expression. Once established, this gene-expression program must be maintained to ensure development proceeds. A major challenge to the maintenance of a robust gene-expression profile is mitosis. During mitosis, the chromatin condenses, transcription arrests, and most transcription factors dissociate into the cytoplasm. Despite these substantial disruptions, the gene expression profile is rapidly and robustly re-established in the daughter nuclei. The essential mechanisms needed to preserve cell identity across mitotic divisions are poorly understood. A subset of transcription factors remains associated with mitotic chromosomes, suggesting that these proteins may function to maintain cell fate. However, for many transcription factors the functional relevance of this mitotic retention is unclear. To better understand how developmental programs are stably propagated through the mitotic divisions, we focus on GAGA factor (GAF). GAF is required in the early *Drosophila* embryo to promote the establishment of both the active and silent genomes. Live-imaging experiments demonstrate that GAF is retained on the mitotic chromosomes, but this retention is developmentally regulated. GAF is evident at the pericentric heterochromatin during the rapid and synchronous early mitotic divisions, but at gastrulation GAF is no longer mitotically retained. Furthermore, GAF retention at euchromatin has been suggested to be instrumental in facilitating memory of the transcriptional state through mitosis. We identified protein domains important for GAF mitotic retention and individual amino acid residues that, when mutated, result in increased mitotic retention. Mutations of these serine residues result in the persistence of GAF mitotic retention into gastrulation. Our data support a model in which phosphorylation of serine residues in the DNA-binding domain weaken the affinity of GAF for chromatin and mutating these residues increases mitotic retention at both heterochromatin and euchromatin. To determine whether this increased retention of mutated GAF changes the gene-expression profile of the embryo, we made endogenous mutations in GAF and will present data on the impact of these mutations. Our studies of the essential transcription factor GAF provide insights into the functional relevance of mitotic retention in maintaining a robust gene-expression program.

449T **Monitoring fatty acid trafficking in follicles reveals a critical role for the triglyceride synthase DGAT1 in protecting mitochondrial integrity** Roger P White, Michael A Welte BIOLOGY, University of Rochester

During Drosophila oogenesis, LDs (lipid droplets) accumulate in nurse cells (NCs), but the mechanism of how lipid metabolism

contributes to follicle development is unknown as these LDs might simply provide energy reserves for the future embryo. To address this question, we treated explanted mid-stage follicles with CPT1 inhibitors to block uptake of long-chain fatty acids (FAs) into mitochondria. We monitored mitochondrial membrane potential (MMP) in NCs using the dye TMRE and found that within 30 mins levels of MMP dropped by nearly half. MMP was also reduced upon germ-line knockdown of several enzymes involved in fatty acid oxidation (FAO). These observations suggest that NC mitochondria rely to a substantial extent on FAO for energy production. Because MMP of NCs is also reduced in mutants for the triglyceride lipase ATGL, we hypothesize that the triglycerides (TAGs) in LDs are the source of FAs for FAO. To directly monitor FA trafficking, we exposed follicles to fluorescently labeled FAs (FLFAs) or fed FLFAs to flies. With *ex vivo* incubation or short-term feeding, fluorescent signal predominately appears in LDs. In mutants for the TAG synthesis enzyme DGAT1, LDs are not produced and FLFAs accumulate in mitochondria. We find that *DGAT1* mutant NC mitochondria display increased levels of mitochondrial ROS and take on a toroidal morphology often associated with oxidative stress. They also are functionally impaired as the mutant follicles display lower oxygen consumption rates and decreased ATP levels. Finally, *DGAT1* mutants arrest in stage 9 of oogenesis. We propose that in the absence of LDs unrestrained influx of FAs damages mitochondria which in turn causes developmental arrest. Indeed, when we throttle mitochondrial FA import with CPT1 inhibitors, mitochondrial ROS is reduced and some follicles advance up to stage 10B. Lipophorin receptors LpR1 and LpR2 are responsible for facilitating the uptake of exogenous lipids into follicles, and we have found that reducing their dosage in *DGAT1* mutants increases the frequency of follicles that advance to stage 10B. To specifically test the role of FA import into mitochondria, we are in the process of analyzing *CPT1 DGAT1* double mutants to see if the morphological and bioenergetic defects are rescued and if follicle progress beyond stage 9. In summary, we find that LDs in NCs are critical for pacing the import of FAs into mitochondria and to protect mitochondria from FA overload.

450T **Heat shock alters nucleolar morphology in *Drosophila* nurse cells** Anna S Ramsey, Danielle E Talbot, Tina L Tootle Biology, University of Iowa

The nucleolus is a membraneless organelle that plays a critical role in cell signaling and has many functions, including ribosome biogenesis, organizing heterochromatin, regulating the cell cycle, and activating the cellular stress response. Using *Drosophila* oogenesis, our lab found inhibition of RNA polymerase I (RNAPI) and loss of prostaglandin synthesis result in two distinct nucleolar morphologies. Nucleolar morphology is deeply connected to nucleolar function; changes in morphology indicate altered function. Therefore, we wanted to explore how other stimuli affect nucleolar morphology and focused on the effects of heat shock. In amniotes, the nucleolus contains three compartments: the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC). It is currently unclear how many nucleolar compartments are present in *Drosophila* nurse cells. To explore this question, we used GFP-tagged protein lines to assess the localization of various nucleolar proteins and to determine how heat shock alters their localization in Stage 10 follicles. We find Fibrillarin (present in the DFC in amniotes) goes from smooth localization throughout the nucleolus to a speckled pattern with heat shock. The extent of the Fibrillarin speckling is time dependent, with longer heat shocks resulting in more penetrant speckling. Similarly, Nopp140 (present in the DFC in amniotes) exhibits speckled staining in heat shocked follicles. Whereas Modulo (present in the DFC and the GC in amniotes) and Nucleostemin1 (present in the GC in amniotes) also localize throughout the nucleolus but exhibit holes inside the nucleolus. These holes decrease in size in heat shocked follicles. These data suggest the presence of two distinct compartments in *Drosophila* nurse cell nucleoli. Understanding the general organization of the *Drosophila* nurse cell nucleoli and the effects of different stimuli, like heat shock, can provide greater insight into the mechanisms of how cells cope with short term environmental stressors compared to chronic stress states associated with diseases such as cancer.

451T **Determining the roles of prostaglandins in regulating nuclear architecture during collective cell migration** Ashley Goll, Tina Tootle Biology, University of Iowa

Cell migration is required for many biological processes including development, wound healing, and cancer. During migration, the cytoskeleton and nucleoskeleton undergo rearrangements. The nucleus is the largest organelle within the cell and can be the limiting factor in the cells' ability to migrate through space restrictive areas. Information about the external environment is relayed through the cytoskeleton to the nucleus and drives alterations to the nucleoskeletal composition. Altering the composition of the nucleoskeleton regulates the stiffness of the nucleus. When there is more Lamin A and Emerin the nucleus is stiffer, while when there is more Lamin B the nucleus is softer. Little is known on how the nucleoskeleton behaves during 3D collective cell migration. To address this, we use Drosophila border cell migration as a model to observe *in vivo*, 3D collective cell migration. We find that Lamin A is present within only the polar cells while Lamin B is in both the polar cells and border cells throughout migration. Emerin behaves dynamically as it is prevalent in the polar cells and border cells at the beginning of migration but becomes restricted to the polar cells by mid-migration. We next asked if the dynamic behavior of the nucleoskeleton during collective cell migration is regulated by prostaglandins (PGs). PGs are short range lipid signaling molecules that are required for on-time border cell migration. We find that loss of PGs increases Lamin A and Emerin prevalence in the border cells throughout migration, which suggest that the nuclei are stiffer and may contribute

to the migration delays. Supporting this idea, overexpression of Lamin A in the border cells results in delayed migration. These findings imply that nuclear stiffness is a limiting factor in border cell migration. Together these results suggest that the transmission of mechanical signaling from the external environment through the cytoskeleton to the nucleoskeleton is regulated by PG signaling and is necessary for 3D collective cell migration.

452T **New models to investigate Transportin-2 (TNPO2) function in Drosophila and neurodevelopmental disease** Lindsey D Goodman[1], Ziyaneh Ghaderpour Taleghani[2], Hugo Bellen[2] [1]Human Molecular Genetics, Baylor College of Medicine, [2]Baylor College of Medicine

We previously showed that de novo mutations in the nuclear-cytoplasmic shuttling protein transportin-2 (TNPO2) are associated with a rare disease involving global developmental delays and neurological features (Goodman et al. AJHG, 2019). Over 25 cases of TNPO2-associated disease have now been identified worldwide. We previously found that this gene is dosage sensitive – too much or too little expression of the fly orthologue of TNPO2, tnpo, causes developmental toxicity in multiple tissues. Furthermore, overexpressing human TNPO2 is mildly toxic and patient-associated mutations alter this toxicity – some mutations are less toxic than the human reference, indicating they are loss-of-function (LOF), whereas some mutations are more toxic, indicating they are gain-of-function (GOF). This previous work relied on overexpression of TNPO2 and provided little mechanistic insight into how mutations cause disease. Here, we are taking several approaches to further our understanding of this disorder using established and novel fly models. First, we developed a novel protein degradation assay using da-GAL4[geneswitch] (daGS) which shows that a mutant TNPO2:p.D156N reduces TNPO2 protein stability in vivo. Despite TNPO2:p.D156N protein being rapidly degraded, we found that it causes strong motor defects and a bang-sensitive phenotype when ubiquitously expressed in adult flies using daGS to avoid developmental toxicity. This is consistent with our previous work indicating that TNPO2:p.D156N is a strong GOF mutation. To investigate TNPO2-mutations in a more endogenous setting, we are now developing a humanized fly model for TNPO2-disease using novel 1xUAS-TNPO2 fly lines that carry only 1 upstream activation sequence (UAS). This system dramatically reduces the amount of protein produced from the UAS-transgene and successfully avoids toxicity caused by overexpressing human TNPO2. We are now assessing if this lower expression of TNPO2 is sufficient to rescue developmental lethality in transheterozygous tnpo CRIMIC/tnpo[Δ11] mutant flies; the CRIMIC cassette express the GAL4 transcription factor that activates 1xUAS-TNPO2. To further probe the mechanism associated with TNPO2 mutations, we are also developing a GFP-based reporter system which will be used to measure TNPO2-cargo shuttling from the cytoplasm into the nucleus in flies and cultured cells. These new models will be used to investigate novel de novo variants in TNPO2 and to expand our understanding of this rare disease.

453T **Characterization of the regulatory roles of phosphatases in endosomal Microautophagy** Laury Lescat[1], Andreas Jenny[1,2] [1]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]Department of Genetics, Albert Einstein College of Medicine

Autophagy, the lysosomal degradation of cytoplasmic proteins, organelles, and pathogens, is a major catabolic pathway conserved during evolution. Autophagy is a constitutive or adaptive process essential for the maintenance of cellular homeostasis and for the response to cellular stress such nutritional, energetic, genotoxic and oxidative stress. Three major autophagic pathways have been described in vertebrates: Macroautophagy (MA), Chaperone-Mediated Autophagy (CMA) and endosomal Microautophagy (eMI). During eMI, proteins in bulk or KFERQ-containing proteins dock at the endosomal membrane to be subsequently internalized into multivesicular bodies in an ESCRT (endosomal sorting complexes required for transport) dependent process. eMI has only recently been identified and its regulation and physiological role are not well understood. Our lab has identified eMI in *Drosophila melanogaster*, using a biosensor containing a KFERQ motif fused to a photoactivable (PA) mCherry. Briefly, the eMI sensor is expressed in a tissue specific manner using the UAS-Gal4 system to monitor eMI activity in pulse chase types of experiments under different stress conditions. MA and CMA are known to be regulated by a series of kinases and phosphates. Thus, we hypothesized that, analogous to MA, and CMA, eMI is regulated by kinases and phosphatases. Through a primary extensive RNAi screening (600 kinases and phosphatases), we have identified 67 potential candidate eMI regulators that we continue to analyze with a particular focus on 5'-nucleotidase (NT5), Inositol polyphosphate 4 phosphatase (INPP4A/B), and inositol polyphosphate-5-phosphatase (OCRL). All of these kinases are known to have a direct or indirect role in the regulation of macroautophagy and the endolysosomal system through PI3K, AMPK-Tor and GSK3signaling pathway. Using nutrient, oxidative, and genotoxic stress, we are investigating their regulatory roles in the regulation of eMI.

454T **Identification and functional analysis of substrates & regulators of starvation-induced endosomal microautophagy in *Drosophila*** Prasoon Jaya, Simone Sidoli, Andreas Jenny Albert Einstein College of Medicine

Autophagy is an evolutionarily conserved cellular stress response that degrades a wide variety of substrates in lysosomes, including oxidized and aggregated proteins, organelles, and intracellular pathogens, and is thus required for cellular

homeostasis and function. Autophagy dysfunction has been linked to a number of diseases, including HIV, cancer, metabolic disorders, liver diseases, and neurodegenerative diseases. To date, three autophagic pathways have been identified: macroautophagy (MA), chaperone-mediated autophagy (CMA), and endosomal microautophagy (eMI). The latter was discovered relatively recently, and its regulation and physiological role are still poorly understood. During eMI, substrates are engulfed into late endosomes either in bulk or selectively for proteins with a targeting KFERQ motif in a process requiring the Endosomal Sorting Complex Required for Transport (ESCRT) machinery. Our laboratory recently discovered that specific types of cellular stress, such as prolonged starvation and oxidative stress, but not ER stress, induce eMI in Drosophila, implying that eMI is required for proper cellular homeostasis. The *in vivo* eMI substrates and cellular processes regulated by eMI, however, are still unknown. To better understand the molecular basis for regulation and the physiological relevance of this pathway in Drosophila, I am using proteomics and transcriptomics to identify endogenous eMI substrates and biological pathways regulated by eMI. To accomplish this, I have used label-free quantitative proteomics to compare changes in the fat body proteome of starved larvae over different time periods (0h, 4h, and 25h), under different (genetically altered) conditions with fed larvae as a control. Additionally, I have used RNA-seq to examine how changes in gene expression correspond to variations in the protein content of the larval fat bodies over time. Our analysis has identified several proteins as potential eMI substrates. Further research will provide a new perspective regarding eMI regulation on the pathogenesis of various diseases.

455T **Talking about Bruno: revealing the code for protein trafficking into nuclear domains** Travis Main, Anton Bryantsev Kennesaw State University

Nuclear domains are membraneless organelles found in the nuclei of eukaryotic cells. The B-body is a nuclear domain that accumulates the protein Bruno (Bru) in developing Drosophila flight muscles. We used the B-body and Bru as a model to study formation of nuclear domains. Specifically, we sought to identify protein sequences that regulate Bru accumulation in B-bodies. We generated various Bru mutants, which were subsequently tested in flies by immunostaining and fluorescence microscopy. We determined that all three RNA-recognition motifs (RRMs) of the Bru protein must be intact to allow interaction with hsr-omega, a scaffolding lncRNA residing in the B-body. In addition, two intrinsically disordered regions (IDR 1&2) drive Bru into protein aggregates. Using the truncation analysis, we identified a 32-aa region within IDR1 that regulates Bru solubility. We proposed that phosphorylation controls Bru solubility, and in a subsequent RNAi screen we identified Doa kinase as a factor controlling Bru affinity toward B-bodies. In summary, protein accumulation at the B-body depends on a combination of RRM and IDR protein domains. RRMs determine accumulation specificity, while IDRs stimulate phase separation and determine Bru's affinity to B-body. Phosphorylation serves as the means to regulate Bru accumulation in B-bodies via IDR modification. Our study provides a detailed mechanistic insight into the formation of nuclear domains.

456T **Unscheduled endocycles disrupt ovarian structure and function and impair female fertility.** Hunter C Herriage, Brian R Calvi Biology, Indiana University Bloomington

Endocycling cells grow and repeatedly duplicate their genome without dividing. Cells switch from mitotic cycles to endocycles in response to developmental signals during the growth of specific tissues in a wide range of organisms. However, the purpose of a developmental switch to polyploid endocycles remains unclear in many tissues. Additionally, there is an increasing appreciation that cells can switch to endocycles in response to conditional signals, such as in wound healing, aging, or cancer, giving rise to a diversity of beneficial or pathological effects. Overall, however, the impact of these unscheduled endocycles on development is underexplored. To address this knowledge gap, I am using *Drosophila* ovarian somatic follicle cells as a model to examine the impact of unscheduled endocycles on tissue growth and function. Follicle cells normally switch to endocycles at mid-oogenesis. I have found that induction of premature endocycling can have drastic consequences for ovarian function and female fertility. Starting endocycles too early disrupted the later endocycle arrest and transition to developmental amplification of eggshell genes in later oogenesis. These females laid eggs with thin shells and resulted in significant embryonic lethality. Further examination of these ovaries revealed an array of pleiotropic defects, including reduced oocyte growth and aberrant follicular epithelial structure. Finally, premature endocycling inhibited the collective cell migration of a special set of follicle cells known as border cells in mid-oogenesis. As border cells are a powerful model for the collective cell migration observed in human metastases, my last result challenges current models that polyploidy enhances migration and metastasis of human cancer cells. Overall, these findings have broader impact by revealing how unscheduled endocycles can disrupt tissue growth and function to cause aberrant development.

457T **Regulation of Spermatogenesis by Notch Signaling** Emma OFlaherty, Jennifer Jemc, Christine Severude, Adriana Soriano Loyola University Chicago

Notch signaling is an evolutionarily conserved pathway that is crucial in fate acquisition and spatiotemporal patterning. The Notch signaling pathway plays a significant role in gonad development and spermatogenesis in the male gonad, but little is known about the specific targets of Notch signaling in the testis. The Drosophila testis contain two populations of stem cells: germline stem cells (GSCs), which produce maturing sperm and cyst stem cells (CySCs), which produce somatic support

cells known as somatic cyst cells. The CySCs and early somatic cyst cells that surround the spermatogonia express traffic Jam (tj), which encodes a MAF family transcription factor. They continue to express Tj in the somatic cyst cells surrounding the spermatogonia through the transition zone, as they begin to express Eya and shift to a late cyst cell fate. Notch signaling is active in the transition zone during this time and is activated by the Delta ligand expressed in the germline. Previous work in our lab data has shown that increased Notch signaling in somatic cyst cells appears to prevent their transition from early to late cyst cell fate, suggesting that Notch signaling is important for this transition and must be downregulated to progress through development. As little is known about the downstream effectors through which Notch signaling functions to promote cyst cell development, RNA sequencing was performed to identify potential targets of Notch. This approach led to the identification of 1619 potential Notch target genes. Further data analysis was conducted using previously published single-cell RNA sequencing data and gene ontology analysis to narrow down this list to 66 genes. We are now testing these potential targets in control, Notch overexpression, and Notch mutant flies using immunohistochemistry, in-situ hybridization and quantitative PCR to build a genetic network for the Notch signaling pathway and expand our understanding of its role in spermatogenesis.

458T **Xport-A functions as a chaperone by stabilizing the first five transmembrane domains of rhodopsin-1** Pedro Domingos ITQB-UNL, NIF 501559094 Rhodopsin-1 (Rh1), the main photo-sensitive protein of Drosophila, is a seven-transmembrane domain protein, which is inserted co-translationally in the endoplasmic reticulum (ER) membrane. Biogenesis of Rh1 occurs in the ER, where various chaperones interact with Rh1 to aid in its folding and subsequent transport from the ER to the rhabdomere, the light-sensing organelle of the photoreceptors. Xport-A has been proposed as a chaperone/transport factor for Rh1, but the exact molecular mechanism for Xport-A activity upon Rh1 is unknown. Here, we propose a model where Xport-A functions as a chaperone during the biogenesis of Rh1 in the ER by stabilizing the first five transmembrane domains (TMDs) of Rh1.

459T **Fine-tuning of Cell-ECM Assembly by Transglutaminase** Dylan Feist[1], Nicole Green[2], Erika Geisbrecht[1] [1]Biochemistry and Molecular Biophysics, Kansas State University, [2]Biology, Cornell College

The *Drosophila melanogaster* myotendinous junction (MTJ) is a unique model to understand how secretion and assembly of the extracellular matrix (ECM) contributes to cell adhesion during development and growth. MTJ formation requires myotube targeting to the appropriate tendon cell followed by the secretion and binding of ECM proteins to transmembrane integrin heterodimers on opposing muscle and tendon cells. This stable network not only links the ECM to the internal actin cytoskeleton, but also transmits tension between muscles and tendons to withstand contractile forces. Thus, knowledge of the molecular composition of the MTJ throughout development is essential to understand how forces vary at the muscle-tendon interface. We performed a targeted RNA interference (RNAi) screen to uncover secreted proteins that are required for cell-matrix adhesion in the contractile muscles of third instar larvae (L3). One candidate that emerged is Transglutaminase (Tg), a protein with known scaffold and crosslinking activity. Therefore, our hypothesis that the crosslinking function of Tg is important for maintaining extracellular adhesion and ECM integrity is exciting and may change current dogma suggesting that integrins are the primary mediator for MTJ stability. RNAi knockdown of Tg in the embryonic tendon cells resulted in smaller muscle attachment sites and occasionally led to fully detached muscles by the L3 stage. Preliminary studies indicate that Tg activity is required as an antibody generated against the epsilon-(gamma-glutamyl)lysine-isopeptide bond can be visualized at muscle attachment sites. Current and future experiments will focus on examining Tg mutants and further characterizing if the crosslinking role of Tg is essential through expression of a catalytically inactive Tg.

460T **Defining the contribution of the dendritic cytoskeleton to critical period closure** Dunham Clark[1], Sarah D Ackerman[2] [1]Cell & Developmental Biology, Washington University in St. Louis, [2]Pathology and Immunology, Washington University in Saint Louis

Neural networks undergo critical periods of heightened plasticity in development, during which neurons undergo structural and functional changes that directly affect the long-term activity of neural circuits. Disruptions in the timing of the critical periods in humans have been linked to a number of neurodevelopmental disorders, including autism and epilepsy. We have identified that *Drosophila* exhibit a motor circuit critical period that peaks in embryonic during development, during which motor neurons undergo activity-driven morphological changes in their dendrites and synapses. Motor plasticity is maximal during late embryogenesis, and is terminated by 8 hours after larval hatching (h ALH). Our previous work demonstrated that as the critical period comes to a close, and as neuronal plasticity wanes, the microtubules stabilize within the dendrites of *Drosophila* motor neurons. This research aims to characterize cytoskeletal dynamics across this critical period, and to test whether microtubule stabilization is necessary and/or sufficient for critical period closure. We are performing optogenetic activation of *Drosophila* motor neurons at 4 h ALH, midway through the critical period, combined with motor neuron-specific manipulation of genes that drive microtubule stabilization (ringmaker, no distributive disjunction, α-tubulin acetylase, and Kinesin-like protein at 10A) or destabilization (spastin, Fidgetin, Katanin 60, and leaky) to address this hypothesis. We expect

that, if microtubule stabilization is required for proper critical period closure, then motor neuron-specific knockdown of microtubule stabilizing genes will extend critical period plasticity at 8 h ALH; conversely, we expect knockdown of microtubule destabilizers to prematurely close the critical period, but have no effect on plasticity beyond 8 h ALH. Overall, this research utilizes a novel critical period model to discern the role of the cytoskeleton in neurodevelopment.

461T **Role of RRP1B in Regulating Cellular Stress Response in Triple-Negative Breast Cancer Metastasis** Wan-Ning Li, Kent Hunter Center for Cancer Research, National Cancer Institute

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that remains a clinical challenge due to the lack of good therapeutic targets. Characterized by quickly growing tumors, high rates of metastasis, and unresponsiveness to therapies, patients diagnosed with TNBC are commonly associated with poor clinical outcomes and high recurrence rates. Evidence has shown that stress in the tumor microenvironment plays a critical role in malignant tumor progression by altering the genomic actions and the cellular responses in cancer cells. However, the molecular mechanism of stress-induced breast cancer metastasis remains unclarified. Here, we have observed that Ribosomal RNA Processing 1B (RRP1B), a breast cancer metastasis susceptibility gene our lab has previously identified, showed a subnuclear protein translocation from nucleolar to nuclear speckles under heat shock stress, implicating RRP1B participates in the stress-induced response in TNBC. To elucidate the mechanism of RRP1B protein trafficking, we first focused on determining the functional protein domains by bioinformatics prediction. Both tools, InterPro and MobiDB, revealed that intrinsically disordered regions (IDRs), peptides that are characterized by a high proportion of hydrophilic amino acids and low sequence complexity that make them unable to form a well-defined structure spontaneously, cover over 60% of RRP1B protein that suggests a potential role of IDRs in protein characteristics. We therefore generated a deletion construct that could express IDR-truncated RRP1B protein in TNBC cells, and the results showed deletion of IDRs leads to an exclusive expression of RRP1B outside the nucleoli, indicating that IDR is involved in the RRP1B subnuclear trafficking. To further investigate the role of RRP1B in the stress-induced processes in TNBC, we performed RRP1B-immunoprecipitation mass spectrometry analysis in MDA-MB-231 cells. Both Ingenuity Pathway Analysis and Metascape analysis revealed the 136 proteins that are shown to have increased binding with RRP1B under stress condition are strongly associated with spliceosomal cycle. Our future studies will focus on elucidating the molecular mechanism involved in the stress-induced action of RRP1B through further characterizing the nature of RRP1B re-localization and studying the role of RRP1B in splicing process that promotes cancer metastasis in TNBC.

462T **Regulation of Cdc42 Protein Levels Impacts a Cell Differentiation Program** Beatriz González, Paul Cullen Biological Sciences, State University of New York at Buffalo

The orientation of an axis of polarity is a critical feature of all organisms. Cells can reorganize their polarity during the cell cycle and in response to extracellular cues. One type of polarity regulatory protein are Rho GTPases. Rho GTPases interact with proteins that control the cytoskeleton and signaling pathways, yet how they function in specific settings remains in many cases unclear. Our work centers around the ubiquitous Rho GTPase Cdc42p, which regulates signaling through evolutionarily conserved Mitogen-Activated Protein Kinase (MAPK) pathways. In the budding yeast *Saccharomyces cerevisiae*, Cdc42p regulates MAPK pathways that govern morphogenetic changes in response to external signals. We recently discovered that Cdc42p is modified by ubiquitin. The active conformation of the protein was preferentially ubiquitinated by the ubiquitin ligase Rsp5p and by heat shock protein (HSP) chaperones for degradation in the 26S proteasome. A GTP-locked (Q61L) and turnover-defective (TD) version of Cdc42p, $Cdc42p^{Q61L+TD}$, hyperactivated the MAPK pathway that regulates filamentous growth (fMAPK). Unexpectedly, $Cdc42p^{Q61L+TD}$ did not impact the activity of another MAPK pathway that shares components with the fMAPK pathway but induces a different response (mating). An SMG-GDS-type adaptor protein for the fMAPK pathway, Bem4p, stabilized the Cdc42p protein, resulting in sustained fMAPK pathway activity. We also identified a residue on Cdc42p (K16) that was required for protein stability. Altering Cdc42p stability led to protein misfolding and the formation of cytosolic aggregates. We also discovered that the main Cdc42p effector in the MAPK pathway, the p21-Activated Kinase (PAK) Ste20p, was degraded by the 26S proteosome. The regulated turnover of Rho GTPases and PAK kinases may broadly impact signaling pathways that execute specific developmental programs.

463T **Understanding the Heterogeneity in Gene Regulatory Responses to Misfolded Protein Toxicity** Rachel Eder, Leandra Brettner, Kerry Geiler-Samerotte Arizona State University

Protein misfolding is a problem faced by all organisms, but the reasons behind misfolded protein toxicity are largely unknown. Pinpointing one exact mechanism is difficult as the effects of misfolded proteins can be widespread and variable between cells. To better understand the impact of misfolded proteins, here we explore their consequences including how they might affect cells heterogeneously. In order to look for cell subpopulations, we built and optimized a cutting-edge single-cell RNA sequencing platform (scRNAseq) for yeast. By using scRNAseq, we can study the expression variability of many genes (i.e., how the transcriptomes of single cells differ from one another). To induce misfolding and study how single cells deal with this

stress, we use engineered strains with an orthogonal protein with varying degrees of misfolding. When we computationally cluster these cells by their sequenced transcriptomes, we see more cells with the severely misfolded protein in subpopulations undergoing canonical stress responses. For example, we see these cells tend to overexpress chaperones and upregulate respiration. Both of these are hallmarks of the "Generalized" or "Environmental Stress Response" (ESR) in yeast. Interestingly, we do not see all components of the ESR upregulated in all cells, which may suggest that the massive transcriptional changes characteristic of the ESR are an artifact of having defined the ESR in bulk studies. Instead, we see some cells activate chaperones, while others activate respiration in response to stress. In sum, by using our novel single-cell approach, we have gleaned new insights about how cells respond to stress, which can help us better understand diseased cells. These results may also teach how cells contend with mutation, which commonly causes protein misfolding and is the raw material of evolution. These results are the first to explore single-cell transcriptional responses to protein misfolding, and suggest that the toxicity from misfolded proteins may not affect all cells' transcriptomes uniformly.

464T **Functional Regulation of a SUMO E3 ligase during the SUMO Stress Response** Mariana VanPelt, Cate Jones, Oliver Kerscher Biology, The College of William & Mary

The SUMO Stress Response (SSR) is conserved from yeast to humans and involves a rapid accumulation of SUMO conjugates throughout the cell. Experimental evidence suggests that this stress-induced sumoylation program maintains integrity of protein complexes and the solubility of denatured proteins prior to degradation. Siz1 and Siz2, conserved SIZ/PIAS-type SUMO E3 ligases in budding yeast, play a central role in the SSR. Double mutants of siz1Δ siz2Δ completely fail to mount a SRR and display a slow growth phenotype. However, Siz1 is the only SUMO E3 ligase with both nuclear and cytoplasmic substrates. In mitosis, Siz1 becomes sumoylated and phosphorylated by an unknown kinase and is transported across the nuclear envelope via the karyopherin Msn5. We previously showed that Siz1 is ubiquitinated and degraded in an Slx5-dependent manner when its nuclear egress is prevented in mitosis. Here we address the question of how Siz1 levels and modification are controlled in the cytosol. Since PIAS1, an orthologue of Siz1 in mammalian cells, is phosphorylated by casein kinase 2, we used a yeast casein kinase (yck) mutant to analyze steady state levels of Siz1 and sumoylated proteins in response to proteotoxic stressors such as heat and oxidizing agents. Additionally, we are presenting work on ubiquitin ligases involved in cytosolic protein quality control after heat shock, such as Rsp5, that may also be involved in Siz1 regulation. In summary, our findings may aid in delineating a regulatory network for Siz1 in the cytosolic SSR.

465T **Visualizing and Quantitating Stress-Induced Cytosolic Sumoylation** Bryant Humphries, Lily Torrans, Dilynne Hsu, Oliver Kerscher Biology, The College of William & Mary

SUMO, a small ubiquitin-like modifier protein, modulates protein function to regulate essential cellular processes, including the response to stress. In yeast and mammalian cells, exposure to stressors such as elevated temperatures or oxidizing agents initiates a wave of sumoylation that propagates throughout the nucleus and cytosol. This SUMO Stress Response (SSR) has been reported to protect protein complex integrity, prevent protein aggregation, and enhance stress-specific transcriptional responses. However, the choreography and functional targets of this stress-induced sumoylation in the cytosol are not well understood. To further understand and quantitate aspects of stress-induced cytosolic sumoylation in yeast cells, we employed a split-GFP approach. Fragments of the green-fluorescent protein are fused to SUMO-targeted proteins (GFP1-10) or anchored to the mitochondrial surface (GFP11). When these tagged proteins interact on the mitochondrial surface they reconstitute a full GFP protein and fluoresce. Our preliminary data show that there is a statistically significant increase in mitochondrial fluorescence after heat stress when Apj1 (a conserved Hsp40 chaperone) is fused to GFP1-10. In contrast, when we fused a Cdc48-interacting protein, Ubx4, to GFP1-10, we observed heat-stress induced changes of mitochondrial morphology. Additional experiments demonstrate that these stress-induced phenotypes are sumoylation dependent. Deletion of the Siz1/PIAS SUMO E3 ligase decreases fluorescence in cells with the Apj1 and Ubx 4 GFP 1-10 constructs. In summary, these data are consistent with an increase in stress-induced sumoylation on the mitochondrial surface. Refining this assay may hold great utility for the study of SUMO-dependent stress-induced protein interactions in the cytosol and the study of genetic pathways that control them.

466T **Investigating the function of Tvp18 and its role in AP-1 mediated intra-Golgi recycling** Jade Bowerman, Richa Sardana Molecular Medicine, Cornell University

The Golgi consists of cisternal compartments that mature and eventually disperse into vesicles at the *trans*-Golgi network (TGN). While many secretory proteins are modified in the Golgi and leave with these vesicles to their final destinations in the cell, the Golgi's resident proteins must be recycled via retrograde vesicular transport to maintain their proper functional location within the Golgi. Because many resident Golgi proteins are modifying enzymes, such as glycosyltransferases, that act on secretory proteins, disruption of this recycling process can have widespread implications for the health of the cell. Although the importance of cytosolic coats in intra-Golgi recycling is well known, much about how they facilitate recycling is yet to be

discovered, including the role of accessory factors that associate with coat proteins. The highly conserved heterotetrameric clathrin adaptor complex AP-1 acts as one of these coats and facilitates recycling of Golgi residents from the TGN to medial-Golgi. AP-1 can exist as two sub-complexes in *S. cerevisiae* (annotated as AP-1C and AP-1R) depending on the incorporation of an alternative isoform of one of the four subunits. Based on mutually exclusive drug sensitivity phenotypes, AP-1C and AP-1R sub-complexes recycle different subsets of Golgi residents. In an effort to understand the distinct function of the AP-1 sub-complexes we compared genetic interaction profiles to find genes that cluster with only one of the sub-complexes, and found that yeast mutants lacking Tvp18, an uncharacterized but conserved membrane protein, highly correlate with subunits of the AP-1R sub-complex of AP-1. In the work presented here, we use genetic interaction analysis, live-cell fluorescence microscopy and biochemical approaches to further characterize the relationship between Tvp18 and AP-1R function. Our preliminary results indicate that Tvp18 likely acts as a transmembrane regulator of AP-1R function. Overall, these findings are important to understand the regulation and distinct functions of AP-1 sub-complexes in intra-Golgi recycling. Furthermore, while only two alternative sub-complexes of AP-1 exist in yeast, humans are expected to express up to 12 distinct sub-complexes due to alternative isoforms of multiple AP-1 subunits. Our findings will elucidate the differential functions of alternate AP-1 sub-complexes and shed light on the conservation of function of Tvp18, and its mammalian homologue Cacfd1.

467T **Humanization of five proteasome assembly chaperones in *Saccharomyces cerevisiae* suggest orthogonal interactions governing proteasome assembly** Homin Jeong[1], Mudabir Abdullah[1,2], Aashiq Kachroo[1] [1]Center for Applied Synthetic Biology, Department of Biology, Concordia University, [2]National Human Genome Research Institute

Proteasome, comprising 19S regulatory particle (RP) and 20S core particle (CP), maintains proteostasis by degrading aging and unwanted proteins to prevent the lethal accumulation of misfolded protein aggregates. 20S CP is responsible for catalyzing substrate cleavage and consists of two outer and two inner rings of 7 distinct α and β subunits.

Complete humanization of yeast proteasome can provide a cost- and time-effective platform for studying mechanisms and diseases associated with human proteasome. While all seven yeast α and 2 of 7 β subunits are humanizable individually, we observe specific pair-wise restrictions, partly resolved by co-humanization of the neighboring interactions. In humans, dimeric complexes PAC1-PAC2 (yeast's Pba1- Pba2) and PAC3-PAC4 (yeast's Pba3-Pba4) assist α-ring assembly while POMP (yeast's Ump1) coordinate subsequent β-ring assembly. We hypothesize that CP subunits collectively generate a larger interface for accessory protein interactions. These distinct orthogonal or diverged interactions may facilitate optimal assembly or sub-cellular localization of the human proteasome. Significant upregulation of yeast assembly chaperones in a partially humanized αCP indicates suboptimal assembly as a likely reason for incompatibility. We seek to fully humanize yeast CP and explore if the co-expression of assembly chaperones is critical.

I used centromeric plasmids to express single and combinations of five human CP proteasome assembly chaperones into yeast. Their expression was insufficient for the phenotypic rescue of partially humanized CP strains. However, co-expression of 5-gene human 20S core assembly chaperones, confirmed via MS, showed synthetic negative interactions only with human α5 and β2 subunits, suggesting interaction prompting further investigation. We hypothesize that the growth inhibition likely occurs from human subunits in hybrid human-yeast CP failing to recruit yeast subunits, resulting in defective proteasome assembly.

In non-quiescent cells, the proteasome serves as a crucial regulator in controlling proteins associated with cell division and DNA repair, by dynamically shuttling between the cytosol and nucleus - process yet to be comprehensively explored. Follow-up studies will investigate if the humanization of the yeast CP affects subcellular localization of the proteasome and identify accessory factors that restore defective localization/assembly of the humanized proteasome in yeast.

468T **Assembly of Polarity Complexes in *Saccharomyces cerevisiae* Spores** Benjamin Cooperman[1], Michael McMurray[2] [1]University of Colorado Anschutz Medical Center, [2]University of Colorado Anschutz Medical Campus

The ability to break symmetry and polarize is vital in ensuring the viability and proper function of many types of cells. As such, the molecular machinery responsible for polarization is heavily conserved between Eukaryotes. This has led to use of the budding yeast *Saccharomyces cerevisiae* as a model system for studying the underlying mechanisms, often in the context of bud site selection and budding itself. In mitotically dividing (vegetative) yeast cells, bud site selection both requires and directs the placement of stable landmark proteins which mark the site of future buds. The Ras-family GTPase Rsr1 module is thought to link these landmark proteins to the cytoplasmic polarity machinery, recruiting downstream effectors to the marked bud site. Unlike vegetative cell division, sporulation does not utilize the budding process, instead relying on meiotic events to generate four *de novo* spores inside of a parent diploid cell. We have shown that that although spores are not formed by budding, the distal pole landmark Bud8 is indeed placed in the spore membrane to direct future buds.

Other than Bud8, we have shown that several other proteins are placed at the polarity spot of mature spores including the septin Cdc10 and Bud5, a GEF for the Rsr1 GTPase module. Interestingly, we found that the Rsr1 GTPase module is not

required to utilize the polarity site as a future bud site upon germination, suggesting a role for Bud8 as a default bud site. In order to understand how the polarity site is built, we used confocal microscopy to interrogate the spatiotemporal dynamics of its assembly. We found that the polarity site is assembled shortly after closure of the prospore membrane. Components of the exocyst as well as the motor protein Myo2 arrive at the polarity site shortly before Bud8 and Bud5, indicating their involvement in the assembly process. Preliminary data suggests that these proteins travel along the cortex of the prospore membrane via actin cables. As Bud8 is an integral membrane protein, we propose that it is first embedded in the membrane of secretory vesicles which are trafficked to the polarity site by Myo2. Once these vesicles reach the polarity site, they can then dock and fuse at a site above the spindle pole body. Although more work must be done to verify and improve our understanding of this process, it is our hope that understanding how spore are able polarize will prove many insights into the systems Eukaryotes have evolved to break symmetry.

469F **UV-vis spectroscopic analysis of four *C. elegans* globin proteins** Anne McAllister, Katherine M. Walstrom Div. Natural Sciences, New College of Florida

Globins are a protein superfamily found throughout many different organisms that are most commonly known as oxygen binding proteins, such as hemoglobin and myoglobin. Globin proteins in *C. elegans* were discovered over a decade ago, but only a few of these 33 globin proteins have been characterized. Three globin proteins, GLB-1, GLB-6, and GLB-26, have been characterized with UV-vis, Raman spectroscopy and x-ray crystallography (Geuens, E. et al., 2010; Yoon, J. et al., 2010). In order to contribute to the limited body of knowledge about *C. elegans* globin proteins, we examined the heme absorbance and oxygen binding capabilities of GLB-10, GLB-21, and GLB-31 under oxygenated and deoxygenated conditions using UV-vis spectroscopy and GLB-1 as a control. Each protein was overexpressed in *E. coli* and purified. Under oxygenated conditions, GLB-1 showed the expected spectrum with a Soret band at 414 nm, and Q bands at 543 nm and at 578 nm. GLB-31 also exhibited these peaks at slightly different wavelengths, with a Soret band at 418 nm and Q bands at 540 nm and 570 nm. Characterizations of GLB-10 and GLB-21 are still ongoing.

Geuens, E., *et al.* BMC Biochem **11**, 17 (2010). https://doi.org/10.1186/1471-2091-11-17.

Yoon, J. *et al.* Biochemistry 49, 5662 (2010) https://doi.org/10.1021/bi100710a.

470F **Co-clustering of nuclear pores and P granules depends on the nucleoporin Nup214 and is not essential in the *C. elegans* germline** Laura L Thomas[1], Geraldine Seydoux[2] [1]Molecular Biology and Genetics, HHMI / Johns Hopkins University School of Medicine, [2]HHMI / Johns Hopkins University School of Medicine

P granules are germline-specific condensates that enrich small RNA pathway components, including Argonautes. In the pachytene region of the *C. elegans* germline, P granules are perinuclear and overlay clusters of nuclear pores (Sheth *et al*, 2010, Development). This “nuage” organization has been proposed to facilitate surveillance of nascent germline transcripts by the small RNA machinery in P granules.

P granule attachment to nuclei depends in part of the P granule protein MIP-1 (Cipriani *et al*, 2021, eLife; Price *et al*, 2021, eLife). Here we report that the non-essential nucleoporin Nup214 also contributes to this organization. In *nup214* mutants, nuclear pores distribute more uniformly across the nuclear envelope, as typically seen in somatic nuclei, and no longer co-cluster with P granules, which accumulate in the cytoplasm away from nuclei. *nup214*;*mip-1* double mutants exhibit an even more severe phenotype with uniformly distributed nuclear pores and few P granules remaining on most pachytene nuclei, suggesting that co-clustering of P granules and nuclear pores is interdependent.

Remarkably, *nup214*;*mip-1* hermaphrodites are fertile at temperatures up to 25ºC and only exhibit sterility and increased germ cell death at 26ºC. These observations suggest that coverage of nuclear pores by P granules is not essential for mRNA surveillance but protects against apoptosis under stress conditions. We will report on our ongoing investigations of how the apoptotic machinery is impacted in *nup214*;*mip-1* mutants.

471F **Glucose-induced developmental delay is modulated by insulin signaling in *C. elegans*** Mary Ladage, Saifun Nahar, Manuel Ruiz, Jose Robledo, Luhua Song, Pamela Padilla University of North Texas

Glucose metabolism is the central pathway for energy production in all cells. While glucose is critically important for cellular functions, it has been shown that excess dietary glucose results in increased glucose concentrations and negative health outcomes in a variety of organisms. Much of the work to understand the effects of excess dietary glucose has been done in relation to aging and disease processes; however, due to global concerns over rising sugar consumption and increases in obesity and type 2 diabetes among children and adolescents, it is of interest to further investigate such effects in the context of development. In this study, we have used genetic, cell biological and transcriptomic methods in the nematode *C.*

elegans as a model to examine the impact of glucose supplementation during development. We show that a glucose-supplemented diet slows developmental progression (termed "glucose-induced developmental delay" or GIDD) and induces the mitochondrial unfolded protein response (UPR^{mt}) in wild-type animals. Mutation in the insulin receptor *daf-2* confers resistance to GIDD and UPR^{mt} in a *daf-16* dependent manner. RNA-sequencing revealed that the transcriptomic profiles of glucose-supplemented wildtype and *daf-2(e1370)* animals are distinct. From this data, we identified a set of 27 genes which are both exclusively upregulated in *daf-2(e1370)* animals fed a glucose-supplemented diet and regulated by *daf-16*, including a fatty acid desaturase (*fat-5)*, and two insulin-like peptides (*ins-16* and *ins-35*). Mutation of any of these genes suppresses the resistance of *daf-2(e1370)* to GIDD. Additionally, double mutation of *ins-16* and *ins-35* in a *daf-2(e1370)* background results in an increase in constitutive dauer formation which is suppressed by glucose-supplementation. Further investigation of the insulin-like peptides revealed that *ins-16* mutation in a wild-type background results in upregulation of *ins-35*and DAF-16 nuclear translocation regardless of diet; however, unlike *daf-2(e1370),* this translocation is not associated with resistance to GIDD. Taken together, these data suggest that glucose-supplemented *daf-2(e1370)* animals maintain developmental trajectory in part through upregulation of specific insulin-like peptide genes and fatty acid desaturation and contributes to a deeper understanding of the mechanisms underlying the resistance of *daf-2(e1370)* animals to GIDD.

472F **HUM-7, a type IX unconventional myosin, is a novel regulator of integrin adhesion complexes in *C. elegans* muscle** Hiroshi Qadota, Stefano Derossi, Jasmine C. Moody, Guy M. Benian Emory University

In vertebrate striated muscle (skeletal and cardiac), much of the force of muscle contraction is transmitted to the outside of the cell via "costameres", which are muscle-specific "integrin adhesion complexes" (IACs; aka focal adhesions). Costameres attach the myofibrils located at the perimeter of the muscle cell to the muscle cell membrane and overlying extracellular matrix and occur at each sarcomeric Z-disk. In the striated muscle of *C. elegans*, IACs reside at 3 locations—the bases of the sarcomeric M-lines and dense bodies (Z-disks) and at the muscle cell boundaries (MCBs). Each IAC consists of the heterodimeric transmembrane protein integrin and many proteins associated with it both intra- and extracellularly. The MCBs contain only a subset of proteins found at dense bodies. In a screen for mutants with defects in the MCB, we identified the gene *pix-1*, which encodes a RacGEF (guanine nucleotide exchange factor) (Moody et al., 2020) and the gene *rrc-1*, which encodes a RacGAP (GTPase activating protein) (Moody et al., in revision). During RacGAP screening, we also found that *hum-7* mutants also show the defect in the MCB. The HUM-7 protein is predicted to have the following domains: An RA (Ras association) domain, a myosin head domain, 4 consecutive IQ domains, 2 C1 (phorbol ester/diacylglycerol binding) domains, and a RhoGAP domain. Based on the sequence of its myosin motor domain and the presence of the other domains, HUM-7 is a class IX unconventional myosin. Two deletion mutants of the *hum-7* show less accumulation of PAT-6 (a-parvin) at MCBs, similar to *pix-1* and *rrc-1* mutants. Since the HUM-7 contains myosin head and RhoGAP domains, we next examined which domain is important for HUM-7's function at the MCB. We investigated missense mutations in the myosin head and RhoGAP domains. Two of three missense mutations in myosin head region show the defect at MCBs, but one missense mutation in RhoGAP domain did not show the defect at MCBs. We prepared an antibody against HUM-7, and localized HUM-7 in muscle cells. By confocal microscopy, anti-HUM-7 antibodies localize between dense bodies and at the MCB but flanking the location of IAC components in body wall muscle cells. This is a fascinating localization pattern and contrasts with the location of PIX-1 and RRC-1 which localize to the M-lines, dense bodies and the MCB. Our working hypothesis is that HUM-7 functions as a transporter of IAC components towards the MCB region.

473F **The CYK4 GAP domain controls contractile ring assembly and dissolution by regulating the cortical targeting of centralspindlin** Aleesa Schlientz[1], Sebastian Gomez-Cavazos[2], Kian-Yong Lee[2], Pablo Lara-Gonzalez[2,3], Arshad Desai[2], Karen Oegema[2] [1]School of Biological Sciences, University of California, San Diego, [2]University of California, San Diego, [3]University of California, Irvine

During cytokinesis, an equatorial contractile ring constricts to partition the cell contents. Contractile ring assembly requires active GTP-bound RhoA generated by the guanine nucleotide exchange factor ECT2. The centralspindlin complex, composed of two molecules of kinesin-6 and two molecules of CYK4, is the major ECT2 activator during cytokinesis. Centralspindlin is phosphorylated by Polo-like Kinase 1 (PLK1) at the central spindle during anaphase and diffuses to the adjacent plasma membrane where it activates ECT2. The N-terminal half of CYK4 is the primary target of PLK1 and engages with ECT2 to activate it, likely by relieving ECT2 autoinhibition, whereas the C-terminal half of CYK4 contains a C1 domain, which contributes to plasma membrane targeting, and a GTPase-activating protein (GAP) domain predicted to interact with a Rho family GTPase. The function of the CYK4 GAP domain has been unclear. Here, we show that the CYK4 GAP domain functions with the adjacent C1 domain to target centralspindlin to the cell cortex. Using an assay we developed to monitor cortical recruitment of centralspindlin during cytokinesis in the one-cell *C. elegans* embryo, we show that RhoA and the GTPase-binding interface of the CYK4 GAP domain are both essential to recruit centralspindlin to the equatorial cortex. Comparison of a CYK4 mutant that disrupts the RhoA binding interface of the GAP domain to a catalytic mutant (R459A) that selectively disrupts the ability of the GAP domain to convert bound RhoA-GTP to Rho-GDP revealed striking differences. In a mutant background partially

compromised for RhoA activation, preventing RhoA binding inhibited furrowing, whereas the catalytic mutant was largely able to complete cytokinesis. In addition, whereas the RhoA-binding mutant reduced centralspindlin recruitment to the equatorial cortex, the catalytic mutant did not. The catalytic mutant additionally seemed to prevent the normal dissolution of cortical centralspindlin and contractile ring components at the end of cytokinesis. These results lead us to propose a model in which cortical recruitment of centralspindlin by the CYK4 GAP domain is central to a feedback loop in which generation of active RhoA at the cell cortex drives further centralspindlin recruitment and RhoA activation to drive rapid contractile ring assembly. As the contractile ring disassembles, conversion of RhoA-GTP to RhoA-GDP by the CYK4 GAP domain is important to ensure timely dissolution of the contractile ring.

474F **Pavarotti and Tumbleweed function in maintaining mitochondrial integrity in post-mitotic muscle tissue** Yungui Guo[1], David Brooks[1], Erika Geisbrecht[2] [1]Biochemistry and Molecular Biophysics, Kansas State University, [2]Kansas State University

The evolutionarily conserved NUAK serine/threonine kinase plays a role in the autophagic clearance of proteins in *Drosophila* larval muscles. While many proteins that comprise the canonical autophagy pathway have been characterized, the identification of new regulators may help understand tissue and/or stress-specific responses. One of the multiple approaches we have taken toward this goal is to compare gene expression levels in control and *NUAK* mutant muscles. We found that the largest class of down-regulated genes are involved in microtubule (MT)-based structure and/or function, consistent with previous data whereby MT organization is altered in *NUAK* mutants. Muscle-specific RNAi approaches were used to knock down candidate genes that affect multiple aspects of MT regulation. Using p62/Ref(2)P/Sequestosome-1 immunostaining as a proxy to assess autophagic flux, Pavarotti (Pav)/kinesin family member 23 (KIF23) emerged as a candidate. Pav and its binding partner Tumbleweed (Tum)/RacGAP1 comprise the centralspindlin complex and are well characterized for their role in cytokinesis. However, the interconnection of Pav and Tum with autophagy has not been explored. The Pav-Tum complex was recently reported to be required for nuclear envelope budding (NEB) in larval salivary gland cells or adult muscles. In *Drosophila* larval muscles, mRNAs necessary for mitochondrial integrity and neuromuscular junction (NMJ) development are components of nuclear envelope buds. New data from our lab show that Ubiquitin and p62 are recruited to damaged mitochondria upon muscle-specific RNAi knockdown of Pav or Tum in larval muscles. Thus, we hypothesize that the loss of mitochondrial mRNAs via NEB reduces mitochondrial function which thereby recruits p62 and associated autophagosome components for eventual degradation of mitochondria in the lysosome. Interestingly, overexpression of Tum or an inactive form of Pav showed the same mitochondrial damage phenotype, suggesting that levels of the centralspindlin complex are important for function. Our results will provide new insight into how cells respond to decreased NEB at the molecular and organismal levels.

475F **Structural and Mechanistic Exploration of Wnt Ligand Maturation** Kate M Henesey, Erica M Selva Biological Sciences, University of Delaware

Wnt signal transduction is fundamental for the development of all animals and is responsible for the homeostasis of renewable cell populations in adults. During development, reduced or absent Wnt signaling results in a wide range of developmental defects and aberrant signaling in adults is recognized as a primary driver of cancers. Strikingly, Wnt signaling is so indispensable to proper organismal function that perturbation or disruption to any player within the Wnt pathways can have serious ramifications, such as osteogenesis imperfecta or colorectal cancer. While the numerous Wnt ligands and target receptors in signal-receiving cells have been extensively studied, the maturation of virtually all Wnt ligands within signal-sending cells is relegated to highly conserved yet understudied components, Porcupine (Por) and Wntless (Wls). Our initial characterization of Por and Wls function within the model organism *Drosophila melanogaster* to produce functional Wnt has informed much of our understanding of Wnt ligand maturation. *Further characterization of the mechanisms by which these players interact to produce mature, active Wnt ligand is essential to better define how Wnt signaling is regulated.*

We discovered that Wls forms homo-dimers dependent upon intermolecular disulfide bridge formation, and these Wls dimers interact with Wnt (2:1, Wls-Wnt). Remarkably, this provides a universal mechanism for Wls dimerization and a possible explanation for the uncharacterized Wnt hand-off between Por and Wls in early ER Wnt processing. My overarching project goal is two-fold: to characterize disulfide bonded Wls dimer interactions in forming Wnt maturation complexes and to characterize disulfide bonded Wls dimer importance in Wnt post-translational modification, secretion, and signaling. We have generated various tagged forms of *Drosophila* Por and Wls wild-type and conserved cysteine mutants were used in co-immunoprecipitation and functional experiments to examine the importance of Wls disulfide bonds in forming early Wnt maturation complexes and Wnt production from the signal-sending cell. **Here, we show that a conserved cysteine amino acid pair within Wls Dimerization Domain 1 (DD1) is required for Wls dimer interaction with Wnt**, and when only one cysteine in the pair is mutated, the total Wnt bound Wls population shifts in favor of dimer formation. Furthermore, conserved cysteines in Wls DD1 are required for proper Wnt N-glycosylation, secretion, and canonical Wnt signaling activity. *This work establishes*

an understanding of how Wls dimers function within a cell to produce functional Wnt ligand, impacting our comprehension of Wnt cellular dynamics, processing, and secretion mechanisms.

476F **Transport of centrioles as cargo ensure proper neural stem cell asymmetric division** Matthew Hannaford[1], Rong Liu[2], Carey Fagerstrom[1], Brian Galletta[1], James Sellers[1], Nasser Rusan[1] [1]National Heart Lung and Blood institute, [2]West Virginia University

Centrosomes are a key microtubule organizing center in the cell that comprise a pair of centrioles surrounded by a matrix of proteins termed the pericentriolar material (PCM). Centrosomes are responsible for the organization of the mitotic spindle, as well as the formation of cilia and flagella. To fulfill these functions both the positioning and microtubule nucleating activity of centrosomes must be tightly controlled. Typically, centrosome positioning is thought to be governed by the activity of microtubule motors, pushing or pulling on the microtubules anchored at the centrosome. In some cell types, centrioles lack PCM and microtubules, and are referred to as inactive centrioles. Inactive centrioles must be motile and their intracellular positioning is critical for asymmetric cell division, yet it remained unknown how centriole motility is achieved and regulated. Our work has revealed that centrioles are microtubule cargo, moving along the microtubule network in a manner involving Kinesin-1. We discovered that Kinesin-1 localized to the outside of centrioles where it interacts with the centrosomal scaffolding protein Pericentrin-Like-Protein (Plp). Through yeast-2-hybrid and an *in vitro* interaction assay we found that Plp interacts with the cargo binding region of the Kinesin-1 heavy chain. Importantly, the specific disruption of this interaction blocked centriole motility *in vivo.* Kinesin-1 is regulated by an autoinhibitory interaction between the motor domain and the cargo binding tail, we have shown using a constitutively active Kinesin Heavy Chain allele that the autoinhibited conformation is unable to interact with Plp for centriole transport. Kinesin-1 autoinhibition can be relieved via the combined activity of microtubule associated proteins such as MAP7 (Ens) or multiple cargo adaptor molecules. We will discuss our recent efforts to combine biophysical methods *in vitro* with *in vivo* genetic analysis to identify the mechanisms relieving Kinesin-1 autoinhibition for centriole transport and asymmetric cell division. In this work we propose the first comprehensive mechanism of how centrioles can move independently of their role as an MTOC, in the context of developing tissue.

477F **Dysregulation of the ER blocks recruitment of centrosome associated proteins resulting in mitotic failure** Katherine Rollins[1], J. Todd Blankenship[2] [1]University of Denver, [2]Biological Sciences, UNIVERSITY OF DENVER

The Endoplasmic Reticulum (ER) is the largest organelle by surface area and makes contact with other organelles throughout the cell. In recent years, the ER's roles within the cell have expanded beyond the canonical roles of calcium storage and protein synthesis. Additionally, the ER has been shown to undergo remarkable morphological transitions throughout cell division. However, whether these changes in ER behavior and morphology modulates mitotic events is less clear. Here, we use the mitotic divisions of the *Drosophila* syncytial embryo to study ER behaviors during mitosis. In a screen for Rab proteins present in the embryo, the ER associated GTPase, Rab1 was identified. Depletion of *Rab1* led to highly penetrant disruption of division cycles along with enhanced buildup of ER at the spindle poles. Interestingly, this excess clustering of the ER is correlated with the formation of a shortened 'mini-spindle' in embryos that are depleted for *Rab1*. Importantly, the recruitment of several key centrosomal proteins at mitotic onset to the spindle poles is weakened after *Rab1* disruption leading to reduced γ-tubulin function, suggestive of a defect in centrosomal maturation. We implicate Dynein in maintaining some ER contact with the spindle poles in embryos and find that dual disruption of *Rab1* and Dynein can rescue maturation defects. We further explore a subset of ER shaping protein's in organizing the ER during mitosis. These results demonstrate that ER levels must be carefully tuned in an organized manor during mitotic processes to ensure proper assembly of the division machinery.

478F **Alternate roles for the actin formin Cappuccino during *Drosophila* oogenesis.** Hannah M Bailey[1], Margot E Quinlan[1,2] [1]Chemistry and Biochemistry, University of California Los Angeles, [2]Molecular Biology Institute, University of California Los Angeles

The *Drosophila* oocyte has long served as a model for understanding oogenesis, the process of egg development. An essential structure in *Drosophila* oocytes is a cytoplasmic actin meshwork that persists during mid-oogenesis. This complex actin network is built by the collaboration of actin nucleators, Spire (Spir) and Cappuccino (Capu). Loss of the actin mesh, and concurrent decrease in Spir and Capu expression, permits the initiation of fast cytoplasmic streaming, thereby mixing nurse cell and oocyte cytoplasmic contents and reinforcing establishment of polarity. Analogous actin meshes, built by Spir and Capu, have been characterized in the mouse oocyte and melanocyte. The function of these networks appears to differ in these two cell types, based on the localization of Capu (the formin). In melanocytes, Capu (FMN1) is cytosolic and contributes to a dispersion mechanism for cargo transport. Yet in mouse oocytes, Capu (FMN2) is found bound to membranes where it is involved in a convergence mechanism to position the mitotic spindle. CapuA, the isoform expressed in the *Drosophila* ovary, appears diffuse, suggesting that Spir, Capu and the mesh they build may function more like the melanocyte than the mouse oocyte. This raises several questions, such as: how does the fly mesh compare to the mesh in the mouse oocyte, how does

it suppress fast streaming, and does it play additional roles? To address these questions, I tested the impact of driving Capu localization to membranes, using the bipartite GAL4/UAS system. I compared the ability of CapuA to rescue a *capu*-null background with that of CapuJ, a testis isoform that contains a myristoylation site – dramatically increasing the membrane localization pattern of Capu. Interestingly, the actin mesh is built and then correctly removed by both isoforms. However, fertility is reduced from 90% for CapuA to 50% for CapuJ. This result suggests that Capu, in addition to building the actin mesh, is important for one or more other processes. Further data suggest that Capu plays a more direct role in anterior/posterior axis establishment than previously thought. Localization of two critical polarity factors, Staufen and MyosinV, are both altered in the CapuJ-expressing egg chambers. This work contributes new insights into *Drosophila* oogenesis. In addition, it demonstrates that subtle changes in the interaction between two actin nucleators leads to formation of actin-based structures that play distinct cellular roles.

479F **The Rho GAP *crossveinless-c* (*cv-c*) plays an important role in caudal visceral mesoderm (CVM) migration during *Drosophila* embryogenesis** Shiva Ahmadi, Jayden Ogbodo, Afshan Ismat Biology, University of St. Thomas

Proper organ formation is dependent upon the migration of cells from one location to another during embryonic development. The intracellular mechanism of actin polymerization which allows cells to move is similar in different cell types and organisms. When a migratory cell receives a signal to move, actin fibers push on the cell membrane which extends out the leading edge (front) of the membrane while the trailing edge (back) retracts. This process of actin polymerization repeats until the cell reaches its destination. One class of proteins that regulates actin polymerization is the Rho GTPases. Rho GTPases are regulated by GEFs (Guanine Exchange Factors) and GAPs (GTPases-Activating Proteins), two classes of proteins that respectively activate and inactivate Rho GTPases. The gene *crossveinless-c (cv-c)* encodes a RhoGAP protein that regulates the GTPase Rho. Caudal visceral mesoderm (CVM) cells are one type of migratory cell that forms the longitudinal visceral muscles which surround the midgut of Drosophila. Currently, we have shown that loss cv-c results in mis-migration of CVM cells and stalled CVM migration. We also show that expressing a constitutively active (CA) version of Rho1 displays an extreme stalled CVM migration. Conversely, expressing a dominant negative (DN) version of Rho1 shows a slightly stalled CVM migration. Continuation of this research will serve to characterize the effects of losing *cv-c* and over-expression of *cv-c* on actin polymerization and CVM migration.

480F **The conserved protein phosphatase inhibitor, Inhibitor-2, regulates the collective cell migration of border cells** Yujun Chen, Jocelyn McDonald Division of Biology, Kansas State University

Collective cell migration is essential in many developmental and pathological processes. In *Drosophila*, border cells travel as a cluster during oogenesis, making them an excellent genetic model for studying mechanisms of collective movement. In our previous study, we demonstrated that Phosphatase 1 (Pp1) catalytic subunits are critical for collective versus single cell behaviors of border cells, but the Pp1 regulatory subunits that mediate these behaviors were poorly characterized. In a recent RNAi screen to identify Pp1 regulatory subunits, we found that the conserved Pp1 regulatory subunit I-2 (Inhibitor-2) is required for border cell collective migration. I-2 is a small intrinsically disordered protein that was originally described as a potent inhibitor of Pp1. Later studies, however, found that I-2 can function as a chaperone as well as an activator of Pp1. Although I-2 has been reported to be involved in diverse cellular processes, its function in collective cell migration is unexplored. Here, we report that loss of I-2 prevents border cell delamination from the follicular epithelium, whereas a weak hypomorphic allele of I-2 delays migration. Live imaging shows that I-2 promotes the correct number and size of cellular protrusions, which help border cells leave the epithelium and migrate. Further, genetic interactions between Pp1 catalytic subunits and I-2 suggest that I-2 behaves as an activator of Pp1 in border cells rather than as an inhibitor. Nek2 (NimA-related kinase), which can form a complex with I-2 and the Pp1 catalytic subunit, may function together with I-2 in border cells as knockdown of Nek2 impairs migration. Overall, our work implicates I-2 as a key regulator of collective border cell delamination and migration through regulation of protrusion dynamics.

481F **An intestinal G Protein-Coupled Receptor regulates metabolism and immunity in Drosophila melanogaster** Daniela Barraza[1], Xiang Ding[2], Bat-Erdene Jugder[3], Paula Watnick[4] [1]Department of Microbiology, Harvard Medical School/Boston Children's Hospital, [2]Infectious Diseases, Boston Children's Hospital, [3]Boston Children's Hospital, [4]Infectious Diseases, Harvard Medical School/Boston Children's Hospital

The intestinal enteroendocrine cell (EEC) senses stimuli through various receptors, including G Protein-Coupled Receptors (GPCRs). Upon activation, EEC GPCRs regulate expression and secretion of enteroendocrine peptides (EEPs) that control important physiological processes including satiety, systemic metabolism, and immunity. We previously found that expression of a previously uncharacterized EEC-specific GPCR, which we temporarily call GPRx, is regulated by the levels of acetate in the intestine. Acetate is a microbiota-derived short chain fatty acid that is known to act through EEC GPCRs in mammals to regulate host metabolism and immunity via production of EEPs. To test the hypothesis that GPRx plays a role in intestinal metabolism and immunity, we knocked down *GPRx* in a subset of EECs that express the EEP Tachykinin (Tk) (Tk>*GPRx*^{RNAi})

and conducted an RNA sequencing experiment. We observed transcriptional downregulation of *Tk, NPF,* and *Dh31*, which are the EEPs expressed in Tk+ EECs. In addition, gene ontology analysis revealed differential expression of lipid metabolism genes. Tk has been shown to regulate intestinal lipid homeostasis and knockdown of *Tk* results in intestinal lipid droplet accumulation. We used fluorescence microscopy to quantify intestinal lipids and the number of cells containing detectable Tk peptide in Tk>*GPRx*^{RNAi} flies. Consistent with our RNA seq results, we observed accumulation of intestinal lipids and a decrease in the number of Tk-expressing EECs. Because EEPs regulate metabolism and energy expenditure beyond just the intestine, we measured resistance to starvation over time and systemic lipids in Tk>*GPRx*^{RNAi} flies after a 3-day starvation period. We observed higher susceptibility to starvation and higher levels of systemic triglycerides (TG) in Tk>*GPRx*^{RNAi} flies, which is indicative of lipid utilization defects in these flies. Finally, since EEC-signaling is known to regulate immunity in the gut, we assessed the impact of GPRx on the response to intestinal infection by orally infecting Tk>*GPRx*^{RNAi} flies with the diarrheal pathogen *Vibrio cholerae*. Interestingly, we found that these flies were more susceptible to infection despite having reduced *V. cholerae* bacterial loads. We are currently exploring the mechanism underlying these seemingly contradictory observations. Overall, our data suggests that intestinal GPRx regulates expression of EEPs, energy utilization during starvation, and the response to intestinal infection.

482F **ArfGAP1 regulates the endosomal sorting of guidance receptors to promote directed collective cell migration in vivo** Alison Boutet[1], Carlos Zeledon[2], Gregory Emery[2] [1]Molecular Biology, Institut de recherche en immunologie et en cancérologie (IRIC), [2]Institut de recherche en immunologie et en cancérologie (IRIC)

Introduction: Collective cell migration plays important roles in morphogenesis, embryonic development and is a main feature of metastasis formation in several cancers. Collective cell migration is characterized by cell-cell adhesion and cell-cell communication. We have previously demonstrated that vesicular trafficking plays a critical role in cell guidance and cell-cell communication during collective cell migration. A screen identified ArfGAP1, a regulator of vesicular trafficking, as important for border cell (BC) migration in Drosophila ovary.

Methods and Results: Between stages 9 and 10 of Drosophila egg chamber development, the so-called BCs form a cluster that is attracted by the oocyte through the secretion of ligands to receptor tyrosine kinases (RTKs). We found that ArfGAP1 loss-of-function induces migration defects, as clusters loose directionality. Further investigations revealed that ArfGAP1 is required to maintain active RTKs at the plasma membrane by inhibiting its sorting in the endolysosomal degradative pathway. Looking further in the degradative pathway, we discovered an increase in Rab7 signal and lysosomes in BCs depleted of ArfGAP1. Our results show that ArfGAP1 is necessary for the proper sorting of active RTKs in endosomes. Moreover, rescue experiments suggest that the role of ArfGAP1 is dependant of its GAP activity and act upstream of Hrs and Lrrk, two regulators of the degradative pathway.

Conclusion and Relevance: We identified ArfGAP1 as a new regulator of BC migration that acts through vesicular trafficking to maintain RTKs at the plasma membrane and promote chemotaxis. This study could reveal a new important mechanism in collective cell migration, and by extent in cancer dissemination.

483F **Investigations into the role of ER in Wolbachia distribution in host somatic tissue.** I›frica Jalai Norman[1], Blake Riggs[1], Ulises Diaz[2] [1]Biology, San Francisco State University, [2]Biology, University of California San Francisco

Insects are major disease vectors, different methods exist for combatting these vectors, but one understudied method is the targeting of the endosymbiont *Wolbachia*. *Wolbachia* is an intracellular alpha-proteobacterium known for infecting most arthropods. It infects most of the host's somatic cells and relies exclusively on the female germline to pass itself on. In addition to this, it's also known for developing symbiotic relationships with certain infected hosts and can grant viral resistance to infected cells. A major question involving *Wolbachia* is how it localizes itself to different parts of the host body during embryonic development, how it distributes itself from cell to cell during the hosts adult phase. Here, we propose to address this question and hypothesize that *Wolbachia* are using a similar mechanism that mitochondria use for distribution around the cell. To examine this, we plan on performing fixed stained and live analysis of *Wolbachia* and the Endoplasmic Reticulum (ER) using confocal microscopy in the early Drosophila embryo. We believe that *Wolbachia* are using ER-mitochondria contact sites for transport and inheritance into daughter cells during division, thereby displacing mitochondria. This is based on preliminary data that *Wolbachia* infected cells displays lower mitochondria levels during division. We will examine the localization of *Wolbachia* at ER-Mito contact sites and disrupt the Rho GTPase Miro, which has been shown to control ER-Mitochondria contact sites in Drosophila. Understanding how *Wolbachia* is able to distribute among somatic cells will deepen our knowledge of cellular organization and provide an intervention strategy towards insect-borne diseases.

484F **Neuronal triglyceride metabolism regulates sex differences in fat breakdown** Colin Miller[1], Jasper Fisher[1], Lianna Wat[1], Sanjana Prakash[1], Niyoosha Yoosefi[1], Serena Hollman[1], Yi Han Xia[1], Romane Manceau[2], Danie Majeur[2], Tao Huan[1], Thierry

Alquier[2], Elizabeth Rideout[1] [1]University of British Columbia, [2]Universite de Montreal

Drosophila females store more fat than males and have slower fat breakdown after nutrient withdrawal. We previously showed that neuronal loss of triglyceride lipase *brummer* (*bmm*) blocked the sex difference in fat breakdown. Because we now reproduce these sex-specific effects in flies with neuronal loss of additional genes related to intracellular triglyceride metabolism and storage, our data suggests that the sex-specific regulation of neuronal triglyceride contributes to male-female differences in body fat. Yet, the underlying mechanism(s) remain unclear, as much remains to be discovered about the intracellular regulation and function of triglyceride in neurons. To address this knowledge gap, we expressed a lipid droplet-targeted GFP within neurons. This allowed us to monitor neuronal lipid droplets, a specialized organelle dedicated to triglyceride storage. We found lipid droplets in neurons under normal physiological conditions and showed age- and diet-dependent changes to neuronal lipid droplets in both sexes. We identified multiple genes, including *bmm,* that regulate the number of neuronal lipid droplets in a sex-biased manner and show profound sex-specific changes to brain lipidome caused by loss of neuronal *bmm*. Given that loss of *bmm* within the adipokinetic hormone-producing cells (APC) reproduced the sex-specific effect on fat breakdown associated with reduced function in these neurons, our findings suggest a model in which triglyceride supports APC function in males. In light of our discovery of a similar male-specific requirement for neuronal ATGL, the mammalian homolog of *bmm,* in regulating whole-body energy homeostasis in mice, our data identifies a previously unrecognized and sex-specific role for triglyceride in supporting the function of metabolic neurons.

485F **ALS8-related endoplasmic reticulum protein Vap33/VAPB is extracellularly secreted via the topological inversion and MMP1/2-mediated cleavage** Mizuki Tando[1], Kosuke Kamemura[1], Rio Kozono[1], Misako Okumura[1], Daisuke Koga[2], Satoshi Kusumi[3], Kanako Tamai[4], Aoi Okumura[1], Sayaka Sekine[5], Daichi Kamiyama[6], Takahiro Chihara[1] [1]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, [2]Department of Microscopic Anatomy and Cell Biology, Asahikawa Medical College, [3]Department of Morphological Sciences, Graduate School of Medical and Dental Sciences,Kagoshima University, [4]Department of Biological Science, School of Science, Hiroshima University, [5]Graduate School of Life Sciences, Tohoku University, [6]Department of Cellular Biology, University of Georgia

VAMP-associated protein (VAP) is a type IV integral transmembrane protein at the endoplasmic reticulum (ER). VAP is required for tethering ER membranes and various intracellular organelles at membrane contact sites to maintain their structure and function. Humans possess two VAPs (VAPA and VAPB). Several mutations (P56S, T46I, etc.) in human *VAPB* have been reported to be associated with amyotrophic lateral sclerosis (ALS). VAPB is composed of an N-terminal MSP (major sperm protein) domain, a coiled-coil domain, and a C-terminal transmembrane domain. In addition to its intracellular functions, the N-terminal MSP domain of VAPB is cleaved, secreted to the extracellular space, and acts as a signaling ligand for cell-surface receptors including Eph and Roundabout. In humans, the VAPB MSP domain is secreted in the cerebrospinal fluid, and its amount was significantly decreased in sporadic ALS patients, suggesting that impairment of VAPB extracellular functions may contribute to the pathogenesis of ALS. Although the extracellular functions of VAPB are beginning to be understood, it is unknown how the VAPB MSP domain facing the cytosol is secreted to the extracellular space. In this study, we use Vap33 (a *Drosophila* VAPB) and human VAPB to investigate the secretion mechanism of Vap33/VAPB. We show that *Drosophila* Vap33 uses the COPI/II-dependent secretion pathway to reach the plasma membrane, where the MSP domain is exposed extracellularly by topological inversion. Similarly, human VAPB is also extracellularly localized by topology inversion in HeLa cells, suggesting that the extracellular localization of the MSP domain of Vap33/VAPB is an evolutionarily conserved phenomenon. We also found that the externalized MSP domain is cleaved by Matrix metalloproteinase 1/2 (Mmp1/2), membrane-bound or secreted proteinases that cleave extracellular proteins. Overexpression of Mmp1 restores decreased levels of extracellular MSP domain derived from ALS8-associated Vap33 mutants. We propose an unprecedented secretion mechanism for an ER-resident membrane protein, which may contribute to ALS8 pathogenesis.

486F **The role of the SR protein 9G8 in the *Drosophila* intestine to regulate lipid metabolism** Roman Voskoboynikov, Justin DiAngelo Penn State Berks

For the last several decades, the rate of metabolic diseases in humans, such as obesity or type 2 diabetes, has been increasing drastically. These diseases arise from defects in the body's ability to take in and store nutrients such as carbohydrates and triglycerides. Previous studies in the fruit fly, *Drosophila melanogaster*, have identified mRNA splicing factors as having an impact on fly lipid storage. Specifically, adipose tissue knockdown of SR proteins, which regulate splice-site selection, results in alterations in triglyceride storage in whole flies. However, whether SR proteins function in other tissues to regulate nutrient metabolism is not known. We focused on studying the role of SR proteins in intestines by decreasing their levels in the fly gut and measuring the concentrations of lipids and glycogen. We explored eight such proteins, including *9G8*, *B52*, *SF2*, *SC35*, *Rbp1*, *Rbp1-Like*, *RSF1*, and *Srp54*. Decreasing *9G8*, *RSF1*, *SC35*, and *Srp54* in the intestine increased triglyceride levels, while decreasing *Rbp1* in the intestine limits triglyceride storage in female flies. Decreasing *RSF1* and *Rbp1-Like* in the intestine also resulted in glycogen to accumulate, while inducing RNAi

towards *Rbp1* and *Srp54* decreased glycogen levels in female flies. We next further investigated *9G8*, an SR protein which displayed a prominent lipid accumulation phenotype when knocked down in the fat body and intestine. To identify the genes that 9G8 regulates to control lipid storage and metabolism in the gut, we performed RNA-Seq on *9G8-RNAi* intestines. Interestingly, decreasing *9G8* in the intestine resulted in increased expression of five fatty acid synthesis/elongation enzyme genes, as well as four triglyceride lipase genes, which may contribute to the triglyceride accumulation phenotype we observed in *9G8-RNAi* intestines. Together, we found that most SR proteins have a unique metabolic phenotype in the fly intestine and specifically *9G8* regulates whole body and intestinal lipid homeostasis by altering the expression of lipid metabolic enzyme genes in the fly intestine.

487F **Differential expression analysis in *9G8-RNAi* fat tissue reveals upregulation of acyl-CoA synthetase genes in *Drosophila*** Erick Astacio, Justin DiAngelo Penn State Berks

Evolutionarily, animals have adapted systems that store excess nutrients for times of starvation. However, due to the abundance of food in Western society, these storage mechanisms can lead to obesity and other metabolic diseases. Genome-wide RNAi screens in *Drosophila* cells have identified several groups of genes involved in triglyceride metabolism and storage. Previous studies from our lab have characterized the metabolic role of a group of splicing factors called SR proteins that function to identify intron/exon borders. Specifically, one SR protein that has been shown to regulate triglyceride storage in fly fat tissue is 9G8; decreasing 9G8 function resulted in an increase in triglyceride storage phenotype. To better understand the mechanism whereby 9G8 affects the storage of excess nutrients as triglycerides, RNA sequencing was performed on flies with decreased levels of *9G8* in the *Drosophila* fat body. A group of genes that was upregulated in *9G8-RNAi* flies and potentially responsible for the increase in triglyceride phenotype were acyl-CoA synthetases. These proteins facilitate an early step of fat metabolism by adding CoA groups to fatty acids for activation. Once activated, these fats are transported to the mitochondria for beta-oxidation or used for triglyceride esterification in the cytosol. Two uncharacterized acyl-CoA synthetase genes in *Drosophila*, *Acsx4* and *Acsx5*, were identified from the RNA-Seq analysis. To assess their function, RNAi knockdown of *Acsx4* or *Acsx5* was performed in the adult *Drosophila* fat body. Decreasing the expression of both *Acsx* genes resulted in a triglyceride accumulation phenotype suggesting that these genes are important for beta-oxidation of fatty acids. These findings indicate that the *Acsx4* and *Acsx5* genes are necessary for regulating lipid breakdown in the fat body of *Drosophila*, and their increased expression may be compensatory for the increased TAGs observed in *9G8-RNAi* flies.

488F **The Intrinsically disordered region of Drosophila Canoe plays a critical role in linking adherens junctions to the cytoskeleton during embryonic morphogenesis** Corbin Jensen, Emily D McParland, Noah J Gurley, Yufei Xiao, Kevin C Slep, Mark Peifer Biology, University of North Carolina - Chapel Hill

It is becoming increasingly apparent that Intrinsically Disordered Regions (IDRs) play key roles in virtually every cellular function, but we're only beginning to dissect their precise molecular roles. Our lab focuses on mechanisms that link cell-cell adherens junctions (AJs) to the actin cytoskeleton. During embryonic development the interactions between AJs and the cytoskeleton are both dynamic, as the embryo and its component cells are constantly changing shape, as well as robust since the cytoskeleton generates a high level of pulling force on cell-cell contacts as the cells change shape and move. Disrupting interactions between AJs and the cytoskeleton disrupts diverse morphogenetic events, from gastrulation to germband extension to dorsal closure. Connections between AJs and the cytoskeleton involve a complex network of proteins. A central member of this network is the Drosophila protein Canoe. Canoe and the mammalian homolog Afadin contain multiple domains, some of which have known binding partners and are well studied, e.g. Rap1-binding RA domains and the E-cadherin-binding PDZ binding domain. However, Canoe and Afadin also contain a large C-terminal IDR, comprising over 1/3 of the total protein. The IDRs of both Canoe and Afadin are quite different in length, charge and amino acids composition, and have little sequence conservation. Several of the regions in the IDR that are most conserved over evolution are predicted to be alpha-helical by AlphaFold, two of which are the only regions conserved between Canoe and Afadin. Combining bioinformatic, genetic and cell biological analyses, we seek to define the function of the IDR on Canoe function and localization during embryonic development. We generated mutants that completely remove the IDR from Canoe *in vivo*. Loss of the IDR in Canoe dramatically reduces function and largely, but not completely, eliminates localization of Canoe to cell junctions. Instead, deleting the IDR results in strong nuclear localization of Canoe. Replacement of the Canoe IDR with that of Afadin restores substantial but not full function. We're currently analyzing mutants that delete different regions of the IDR, to determine the balance of function of unstructured regions and the two conserved helices. This in-depth analysis of the IDR will provide a clearer picture of how different sequence features of IDRs help Canoe maintain the interaction between junctions and the cytoskeleton, and thus drive embryonic development.

489F **Identifying neurons in which Glut1 acts to regulate nutrient storage in *Drosophila*** Prem Patel, Katelyn Acrie, Justin DiAngelo Penn State Berks

Metabolic homeostasis is regulated by many genes, and defects in some of these genes result in metabolic diseases, such as obesity and diabetes. Several genetic screens have been performed in *Drosophila* to identify genes important for lipid and carbohydrate storage. A gene identified in one of these screens is *Glut1*, a gene that codes for a glucose uniporter in *Drosophila*. Previous studies in our lab have identified a metabolic function of *Glut1* in the fly brain; however, the specific neurons in which *Glut1* acts to regulate nutrient storage are not known. To determine the neuronal populations in which Glut1 acts to regulate lipid and carbohydrate storage, we decreased *Glut1* levels in specific neurons, and measured triglycerides (TAGs), glycogen, and glucose levels. Inducing RNAi to *Glut1* in all neurons resulted in a decrease in TAG and glycogen for both males and females. We next focused on a specific group of neurons expressing the corazonin gene, *Crz*, a neuropeptide that has been shown to be involved in glucose and lipid metabolism by playing a role in regulating insulin-producing cells. We used a *Crz-Gal4* driver to knockdown *Glut1* specifically in the *Crz*-expressing neurons and measured TAGs and glycogen. Inducing RNAi to *Glut1* in *Crz* neurons resulted in a decrease in glycogen in males, but not in females, while there was no change in glucose and TAG in either sex. These data suggest that Glut1 acts in the *Crz* neurons to regulate carbohydrate homeostasis within male *Drosophila*.

490F **Effects of alterations in vesicular acetylcholine transporter expression on acetylcholine homeostasis in the central nervous system** Rohina A. Nemat, Benjamin A. Church, Hakeem O. Lawal Biological Sciences, Delaware State University

Acetylcholine neurotransmission is necessary for the regulation of essential life functions such as locomotion and cognition. As a result, increases or decreases in neuronal cholinergic signaling lead to an impairment in learning and memory, and normal locomotive functions. Although much about how acetylcholine is regulated is known, the mechanism through which changes in cholinergic signaling effects changes in ACh-linked behavior is not fully understood. The vesicular acetylcholine transporter (VAChT) mediates the packaging and transport of acetylcholine (ACh) for exocytotic release. However, the manner through which this process occurs and is regulated is not fully understood. Here we use both an overexpression of VAChT (using a construct that we have reported on previously) and mutants in *Vacht* that cause varying decreases in the gene's expression, as tool to increase or decrease (respectively) the amount of ACh released into the synaptic cleft. And we are measuring the effect of that altered state on synaptic activity using a biochemical approach. We have optimized an assay that allows us to reliably measure cholinergic pathway components ACh and choline from as little as ten *Drosophila* heads. Using this assay, we report the surprising data that while VAChT overexpression does not increase total head ACh levels, there is an increase in choline levels, suggesting that there are homeostatic consequences for changes in VAChT expression. We also present preliminary findings from similar experiments with *Vacht* mutants that have reduced transporter expression. Importantly, we report the effects of increase or decreases in *Vacht* expression of the distribution of VAChT to synaptic vesicles relative to other subcellular compartments using super resolution microscopy. Taken together, these data provide important insight into the consequences that follow a disruption in VAChT expression and advance our understanding of how the vesicular acetylcholine transporter mediate the exocytotic release of ACh in *Drosophila*.

491F **Cell-specific genetic manipulation of Drosophila sallimus severely impacts muscle and motor-neuron morphology and physiology.** Andrew H Michael, Tadros A Hana, Veronika G Mousa, Kiel G Ormerod Biology, Middle Tennessee State University

Skeletal muscle allows animals to produce movement, facilitating a robust set of behaviors and interaction with our environment. The ability of skeletal muscles to contract is derived from the unique genes and proteins that are expressed within muscles, most notably thick and thin filaments, and elastic proteins. Within *in vivo* systems investigations of these proteins are particularly difficult as they often lead to gross phenotypic changes, compensatory mechanisms, or lethality. To circumvent this limitation, Drosophila biologists exploit the Gal4/UAS system to selectively express transgenic manipulations in a subset of cells. Here we investigate the role of the sallimus (sls) protein, which encodes a homologue of titin, in muscle development and function at the larval neuromuscular junction. RNA interference (RNAi) of sls using a ubiquitously expressed muscle driver caused embryonic lethality. We next screened for muscle drivers that express in subsets of larval body wall muscles and identified a driver which expresses Gal4 only in muscle fiber 12 (MF12). Knocking down sls using MF12-Gal4 did not impact larval viability. Immunostaining for elements of the sarcomere revealed significant changes in the structure of MF12. Surprisingly, muscle-specific knockdown of sls in MF12 also resulted in drastic changes in synaptic morphology. We also reveal dramatic changes in the size and number of nuclei in the affected muscles. Using our muscle force transducer system, no obvious changes in whole larval force production were observed. Changes in larval crawling are currently being explored. Mutations in human titin are known to cause cardiomyopathies. Dilated cardiomyopathy is associated with mutations in genes encoding sarcomere proteins, with mutations in titin being the most frequent. This disease is found in ~ 1/ 20 000 individuals but causes 10,000 deaths and 46,000 hospitalizations yearly in the United States. Dilated cardiomyopathy is the most common reason for heart transplantation and the third most common reason for heart failure. Despite the prominence and impacts of these diseases, *in vivo* model organism studies of these elastic protein defects are minimal due to the associated lethality. This

work in *Drosophila* provides a potential *in vivo* model for investigations of titin, other elastic proteins, and of muscle proteins generally.

492F **Addressing the role of endosomal Microautophagy in health and diseases** Satya Surabhi[1], Andreas Jenny[2] [1]Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]1.Department of Developmental and Molecular Biology; 2.Department of Genetics, Albert Einstein College of Medicine

Autophagy is a catabolic process that degrades damaged organelles and aggregate-prone proteins and thus is essential for development and cellular homeostasis. It is induced in response to different stressors including starvation, oxidative stress, and accumulation of misfolded proteins. As such, it counteracts various human diseases, and its reduction leads to aging-like phenotypes. Of the three major forms of autophagy, Macroautophagy can degrade organelles or aggregated proteins, and Chaperone-mediated autophagy is specific for proteins containing KFERQ-related targeting motifs. Endosomal Microautophagy (eMI), is a form of autophagy during which substrates are taken up into multivesicular bodies for degradation in a KFERQ-specific manner or in bulk.

Among the three forms of autophagy, the physiological role of eMI is poorly understood. Using a KFERQ-tagged fluorescent biosensor, we are characterizing the physiological role of eMI with a focus on what types of cellular stress activate eMI. Our data suggest that oxidative stress and DNA damage, but not ER stress can elicit an eMI response in an ESCRT machinery-dependent manner, implying a stress-selectivity of the process. Further, we are trying to understand the mechanism of stress-induced eMI by identifying novel regulators of the eMI pathway. We have identified Wiskott-Aldrich Syndrome Protein (WASp) as an eMI regulator that alters the kinetics of eMI induction, as its overexpression results in faster eMI induction in a Cdc42-dependent manner.

Since targeting autophagic pathways has been proposed as a treatment strategy, particularly in non-dividing neurons, we currently are testing the effect of boosting eMI with WASp on the degradation of faulty proteins in fly models of human neurodegenerative disease. We anticipate that the physiological role of eMI in stress regulation and neurodegeneration is conserved in humans.

493F **The effect of loss of ER membrane complex subunit 4 (EMC 4) on life history traits in *Drosophila melanogaster* is cell type-dependent** Otoha Tatami, Maria Jose Orozco Fuentes, Inés Riojas, Rebecca Delventhal Lake Forest College

The endoplasmic reticulum (ER) is involved in the modification, packaging, and insertion of membrane proteins in the cell. The ER membrane protein complex (EMC) is composed of 8-10 subunits that work together to facilitate protein biogenesis. Through a previous RNAi screen, our lab observed that fruit flies (*Drosophila melanogaster*) with a knockdown of the EMC subunit 4 in glial cells resulted in delayed development and a very short lifespan of 5-6 days instead of the normal 2-3 months. We wondered whether EMC4 plays an important role in other types of cells, so we conducted a small screen knocking down EMC4 in different cells of the fly's brain and body utilizing the UAS-Gal4 system. We knocked down expression of EMC4 in all cells, all neurons, subtypes of neurons (insulin-producing and circadian regulatory neurons), a subtype of glia (astrocytes) as well as hemocytes and fat body cells. Knockdown of EMC4 in some cell types (all neurons and insulin-producing neurons), but not all, resulted in a similar developmental delay as seen with the glial knockdown. We also found that the strength of knockdown appears to have an impact, as a weak pan-neuronal driver resulted in a developmental delay, yet the knockdown with a stronger pan-neuronal driver was pupal lethal. Knockdown in none of the other cell types led to as severe of a decrease in lifespan as in glia, but a knockdown in circadian regulatory neurons led to a lifespan of 23-28 days. Interestingly, EMC4 knockdown in some cells resulted in sex-specific increases in their lifespans. Taken together, these findings suggest that EMC4 plays different roles in different cell types and that the loss of EMC4 in some cell types can affect organismal phenotypes such as lifespan and developmental timing. Future research will focus on exploring how EMC4 knockdown affects specific cell functions by measuring phenotypes tied to the expected role of that cell type in *Drosophila*. This will include testing starvation sensitivity of flies with EMC4 knockdown in insulin-producing or fat body cells and measuring circadian activity of flies with knockdowns in circadian regulatory neurons. Overall, our research highlights the potential importance of the role of EMC in producing membrane proteins, which are crucial for many aspects of the nervous system.

494F **Trehalose accumulation rescues altered Ca^{2+} homeostasis phenotypes of the *S. cerevisiae pgm2Δ* mutant** Micaiah M. Wetzold, Niki M. Hamraei, Kelly A. Hernandez, Lara I. Shehadeh, Shruti V. Raghavan, Zoya Waheed, Katherine E. McBroom, Shreya Uppala, Sarah J. Smith, Akshaya Selvamani, Rebecca J. McDonald, Pranavya M. Manickavelu, Krishna Patel, Katrina Ngo, Ashley Charales, Vana Bahram, David P. Aiello Biology, Austin College

Phosphoglucomutase (*PGM*) interconverts glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P) in *Saccharomyces cerevisiae*. Yeast lacking *PGM2*, the major isoform of *PGM*, exhibit slow growth, calcium homeostasis defects, increased G1P accumulation, and a hyperaccumulation of glycogen when metabolizing galactose as a carbon source. Previous work in the

lab demonstrated that increased trehalose synthesis resulting indirectly from *GPH1* overexpression has a rescue effect on the *pgm2Δ* mutant. It is hypothesized that the partial rescue of *pgm2Δ* mutant phenotypes is due to the protective effect of increased trehalose levels in response to the stress induced by the *pgm2Δ* mutation. To examine the effect of trehalose accumulation on the calcium homeostasis defects in the *pgm2Δ* mutant, we both increased and decreased trehalose presence in *pgm2Δ* mutants metabolizing galactose. Trehalose hydrolysis is predominantly driven by two enzymes, acid vacuolar trehalase, and neutral cytosolic trehalase, encoded by *ATH1* and *NTH1,* respectively. *ATH1* and *NTH1* were each knocked out and overexpressed in the context of the *pgm2Δ* mutation. Loss of *ATH1* function rescues the growth sensitivities associated with the *pgm2*Δ mutant. Alternatively, the overexpression of *ATH1*, which promotes vacuolar trehalose hydrolysis, exacerbates the *pgm2*Δ growth defects. Furthermore, the overexpression of the neutral cytosolic trehalase, *NHT1*, exacerbates the growth defects associated with the *pgm2*Δ mutant to a lesser extent when compared to *ATH1* overexpression. This may indicate that vacuolar trehalose plays a larger role in the rescue of the *pgm2Δ* mutant. Our results support the hypothesis that trehalose accumulation has a protective effect in the *pgm2*Δ mutant.

495F **Altered reserve carbohydrate metabolism rescues altered growth phenotypes of the *S. cerevisiae pgm2Δ* mutant** Niki M. Hamraei, Micaiah M. Wetzold, Kelly A. Hernandez, Luke T. Wild, Lara I. Shehadeh, Shruti V. Raghavan, Zoya Waheed, Katherine E. McBroom, Shreya Uppala, Sarah J. Smith, Akshaya Selvamani, Rebecca J. McDonald, Pranavya M. Manickavelu, Krishna Patel, Katrina Ngo, Ashley Charales, Vana Bahram, David P. Aiello Biology, Austin College

Phosphoglucomutase (*PGM*) is responsible for interconverting glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P) in *Saccharomyces cerevisiae*. Yeast lacking *PGM2*, the major isoform of *PGM*, exhibit slow growth, calcium homeostasis defects, and an accumulation of glycogen when metabolizing galactose as a carbon source. While decreasing glycogen accumulation has little effect, increasing glycogen breakdown by overexpression of *GPH1*, encoding glycogen phosphorylase, partially rescues *pgm2Δ* mutant defects. This rescue due to *GPH1* overexpression is also accompanied by an increase in trehalose levels in the cell. The nonreducing disaccharide trehalose is known to have protective properties in yeast cells undergoing a variety of stress responses. We hypothesized that the partial rescue of *pgm2Δ* mutant phenotypes is due to the protective effect of increased trehalose levels in response to the stress induced by the *pgm2Δ* mutation. Indeed, we show that overexpression of trehalose-6-phosphate synthase 1 (*TPS1*) increases trehalose levels and rescues *pgm2Δ* growth defects. Alternatively, the *tps1Δ* mutant exacerbates *pgm2Δ* mutant phenotypes, supporting the hypothesis that increased trehalose can suppress *pgm2Δ* growth defects. To further test this hypothesis, we investigated the roles of *ATH1*, encoding acid vacuolar trehalase, and *NTH1*, encoding neutral cytosolic trehalase, in maintaining cellular levels of trehalose in the *pgm2Δ* mutant. Our data further supports the hypothesis that trehalose accumulation has a protective effect in the *pgm2*Δ mutant.

496F **In vivo Proximity Labeling Identifies New Functions for the Lifespan and Autophagy-regulating Kinase Pef1, an Ortholog of Human Cdk5** Haitao Zhang[1], Dong-Mei Zhang[2], Belinda Willard[2], Kurt W Runge[1] [1]Inflammation and Immunity, Cleveland Clinic Lerner Research Institute, [2]LRI Research Core Services, Cleveland Clinic Lerner Research Institute

Cdk5 is a noncanonical cell division kinase important to the terminal differentiation of cells in the nervous system, musculature and kidney. Human cdk5 can substitute for its ortholog in various fungi, showing that cdk5, its cyclins and their activities are conserved across evolution. We previously identified the *Schizosaccharomyces pombe* ortholog of cdk5, Pef1, as regulator of chronological aging and autophagy. To better understand cdk5 function, we used proximity labeling in *S. pombe* to identify proteins that may transiently interact with or bind to Pef1. The APEX2 system, which is activated to biotinylate proteins within an ~10 nm radius by the addition of 1 mM H_2O_2 for 1 minute, was chosen for its short labeling times and robust user-initiated biotinylation. Labeling was optimized by partially digesting the cell wall to increase uptake of the biotin-phenol substrate for the reaction. We also found that immunodepletion of the endogenous biotinylated proteins (pyruvate carboxylase and acetyl CoA carboxylase) from cell extracts did not markedly improve detection of APEX2 labeled proteins.

The APEX2 ORF was fused to the end of the chromosomal Pef1 gene, and proximity labeling was followed by purification of the biotinylated proteins and identification by mass spectroscopy. Of the 255 high-confidence Pef1 neighbors identified in growing cells, one was the 14-3-3 protein Rad24, a regulator of the DNA damage response. The Pef1-Rad24 interaction was validated by reciprocal proximity labeling and co-immunoprecipitation. Deleting the *rad24*$^+$ gene caused the expected reduction in growth on medium with DNA damaging agents, while cells lacking both Pef1 and Rad24 grew better under these conditions, indicating partial rescue of the DNA damage sensitivity. Thus, proximity labeling revealed a novel function for Pef1 in DNA repair. To monitor how Pef1 protein neighbors change under different conditions, we have applied this approach to cells starved for nitrogen to induce autophagy. We identified 177 high-confidence Pef1 neighbors in autophagic cells, 80 of which were not detected in growing cells. GO slim analysis of the Pef1 neighbors' biological functions identified proteins in two processes required in autophagy: regulating actin dynamics and vesicle-mediated transport. These data suggest that Pef1 may regulate autophagy through these two processes.

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497F **Characterizing the role of *YDL206W* in *Saccharomyces cerevisiae*** Leah Ding, David P Aiello Biology, Austin College

In *Saccharomyces cerevisiae*, the enzyme phosphoglucomutase (PGM) conducts interconversion between the metabolites glucose-1-phosphate (G1P) and glucose-6-phosphate (G6P). The gene *PGM2* encodes for the major isoform of the enzyme. Previous research has shown that galactose-grown cells lacking *PGM2* exhibit a slow-growth phenotype and an altered ratio of G1P to G6P in comparison to galactose-grown wildtype cells. The *pgm2Δ* strain also exhibits defects in calcium ion homeostasis, including increased calcium uptake, elevated intracellular calcium accumulation, sensitivity to high extracellular calcium levels, and sensitivity to the calcineurin inhibitor cyclosporin A. Previous research using RNA-Seq and DESEQ-2 analysis identified that the loss of *PGM2* altered gene expression globally. One of the genes that displayed elevated expression levels in *pgm2Δ* was *YDL206W*, an uncharacterized ORF with no direct characterization study until recently. Previous work suggests that *YDL206W* encodes a Na+/Ca2+ exchanger (NCX) protein localized to the vacuolar membrane. It was also categorized within the Calcium Cation Antiporter (CaCA) Superfamily, along with its human ortholog *SLC8B1*. This study aims to characterize the role of *YDL206W*, the localization of its encoded protein, and its relation to *pgm2Δ* growth phenotypes and calcium homeostasis defects. By investigating whether *YDL206W* contributes to the calcium homeostasis defects of *pgm2Δ*, this study could elucidate whether *YDL206W* would function as a model gene for *SLC8B1* to better understand how *SLC8B1* regulates calcium ion homeostasis in humans.

498F **Molecular Logic of TORC1 regulation through Gtr1/2 and Pib2** Andrew Capaldi University of Arizona

The Target of Rapamycin kinase Complex 1 (TORC1) is the master regulator of cell growth and metabolism in eukaryotes. In the presence of pro-growth hormones and abundant nutrients, TORC1 is active and drives growth by stimulating protein, ribosome, lipid, and nucleotide synthesis. In contrast, when hormone or nutrient levels drop, TORC1 is inhibited, causing the cell to switch from anabolic to catabolic metabolism and eventually enter a quiescent state.

Numerous proteins and pathways have been shown to regulate TORC1, including: (i) the small GTPases Rag A/B and C/D and the associated GTPase Activation Protein (GAP), GATOR1/2; (ii) Rheb and the Tuberous Sclerosis Complex; (iii) AMPK; (iv) the Nemo-like kinase; (v) PKA; and (vi) Arf1. However, it remains unclear how the proteins/pathways listed above (and others) cooperate to control TORC1.

Here, to address this question, we examine signal integration at TORC1 in the simple model organism, *Saccharomyces cerevisiae*. Previous work in *S. cerevisiae* has shown that amino acid and nitrogen signals are transmitted to TORC1 via: (1) a heterodimeric pair of small GTPases that are homologous to RagA/B and RagC/D, called Gtr1 and Gtr2. (2) The phosphatidylinositol 3-phosphate binding protein, Pib2. But how and why do Gtr1/2 and Pib2 work together to regulate TORC1?

In this study, we examine the impact that Gtr1/2 and Pib2 have on TORC1 signaling across the proteome, using a combination of phosphoproteomics and standard reporter assays. The resulting data show that Gtr1/2 and Pib2 are both required for full TORC1 activation. Importantly, however, deletion of Gtr1/2 or Pib2 only blocks signaling to a subset of the TORC1 substrates—primarily those involved in amino acid metabolism and nutrient transport. These observations lead us to propose a new model where partial starvation triggers metabolic reprograming via TORC1 (by inactivating Gtr1/2 or Pib2) but does not block cell growth. And in follow up experiments, we confirm that this is, indeed, the case. Specifically, we show that when yeast are first transferred from medium containing a high quality nitrogen source, to medium containing a low quality nitrogen source, TORC1 is completely inhibited to block growth and activate metabolic reprogramming. Then, as the cells adapt to the low-quality nitrogen source, Pib2 is turned on again to re-initiate growth, while Gtr1/2 remains mostly off to ensure that the cells continue to activate the metabolic pathways and transporters necessary for adaptation/survival.

Thus, the TORC1 circuit in yeast uses two different amino acid/nitrogen signaling proteins to drive the cell into a rapid growth state, an adaptive growth state, or a quiescent state, depending on the environmental conditions. We argue that other signaling pathways probably work in a similar way, but multi-level responses tend to be overlooked since most studies follow signaling to a single reporter protein.

499F **Studying the Mechanism for Ribosome-Inactivating Protein-Induced Apoptosis** Daniel Judge West Virginia University

Apoptosis, a conserved process of programmed cell death, can be activated through diverse cellular mechanisms, many of which remain poorly understood. This study focuses on a specific mechanism triggered by ribosome-inactivating proteins (RIP), a category of potent toxins such as ricin and sarcin. Ricin halts translation by irreversibly damaging large ribosomal subunit rRNA through an adenine N-glycosidic cleavage referred to as depurination. While the role of the catalytic subunit of

ricin, known as ricin toxin A chain (RTA), is well-established, the precise process that initiates apoptosis in RTA-compromised cells remains unclear. Importantly, while ricin induces apoptosis, translation inhibitors employing mechanisms other than depurination, such as the peptidyl transferase inhibitor anisomycin, often only induce cell cycle arrest. Ribosome depurination may be a critical step in initiating apoptosis. We hypothesize that ribosomes translating on the same mRNA would collide with the stalled depurinated ribosome resulting in improper recycling when translation terminates. The resulting physical damage to ribosomes would activate ribosome quality control mechanisms, ultimately leading to apoptosis. Disomes resulting from random or induced ribosome collisions activate the integrated stress response. In this study, we will utilize an *in vivo* ricin reporter plasmid to modulate RTA gene expression and examine the apoptotic response in *Saccharomyces cerevisiae*. Previous studies have used galactose-inducible promotors, requiring yeast to be grown on an alternative carbon source that represses ribosome biogenesis. We will use a tetracycline-regulatable promoter novel to RIP studies in yeast, allowing for growth on traditional dextrose media. We will closely monitor ribosomal biogenesis, localization, and degradation before and during apoptosis and compare the effects of wildtype RTA to catalytically dead mutants such as E177K, S215F, and P95L, which do not induce apoptosis. Genetic screens will be conducted to identify the essential elements required for ricin-induced apoptosis, and in-lab evolution experiments will be carried out to identify mutations that confer resistance to ricin-induced apoptosis. Long-term, this research aims to provide a comprehensive understanding of the cellular pathways affected after exposure to RTA and establish a controlled method for reliably inducing apoptosis in specific target cells. The insights gained from this study are likely to have broader implications in the fields of cancer biology, embryology, and immunology, offering new avenues for exploring cellular homeostasis and understanding how cells respond to stress.

500F **Maternal Regulation of Microtubule Organizing Centers in the Egg and Oocyte** Allison Marvin[1], Ruth Cisternas[2], Ronald Heller[2], Ricardo Fuentes[2], Mary Mullins[1] [1]University of Pennsylvania, [2]Universidad de Concepcion

The centrosome is a vital organelle that serves as the main microtubule organizing center (MTOC) of the cell. One of its most crucial functions is to duplicate and form a bipolar spindle structure for chromosome division in mitosis. The faithful division of genetic material is likewise important in meiosis, but the female gamete comprises a special case of microtubule (MT) organization. In early oogenesis, the oocyte disassembles its centrosome. This elimination of the maternal centrosome is conserved across most metazoans and protects the zygote from early aneuploidy. Despite its conservation, maternal centrosome elimination remains poorly understood. In the absence of centrosomes, several noncanonical MT organization strategies have been identified in model species and humans, including acentrosomal MTOCs (aMTOCs). The mechanisms by which oocytes control noncanonical MTOCs spatially and temporally remain obscure. Thus, MTOC regulation during oogenesis is not well-understood, despite its function to prevent aneuploidy in development. We performed a large scale forward genetic screen to identify maternal factors required for early development. This screen generated a maternal-effect mutant whose offspring exhibit numerous ectopic aster-like MT formations and fail to complete the first cell division. This work aims to determine if the ectopic structures are centrosomes or aMTOCs, which will establish the function of the gene mutated as a mediator of centrosome elimination or as a negative regulator of aMTOCs during oogenesis. Our vertebrate model will advance understanding of MT organization in oogenesis and in the egg. Given the importance of proper MT organization for human fertility, this work will address knowledge gaps in the advancement of fertility treatments. Research reported in this abstract was supported by the National Institute of General Medical Sciences of the National Institutes of Health under award number R35GM131908 and in part by T32 HD083185.

501F **Reactive oxygen species (ROS) relax wounds to allow healing and regrowth.** Chang Ding Purdue University

The reactive oxygen species (ROS), pivotal secondary messengers, promote wound healing and tissue regeneration. However, whether ROS trigger wound closure by regulating wound mechanical behaviors remains unknown. Using pharmacological inhibitors and genetic modifications, we observed that ROS relax wound contraction. ROS production-inhibited groups exhibit prolonged wound contraction periods after wounding and impaired tailfin regrowth two days post-amputation. Notably, DPI-treated groups show delayed wound closure 30 mins after wounding, while the control group's epithelia mechanically restore their barrier function. To further delineate the underlying mechanisms through which ROS regulate wound dynamics, we employed inhibitors targeting Rho-associated coiled-coil-containing protein kinase (ROCK) and the myosin light chain kinase (MLCK). The ROCKOUT-treated groups exhibit reduced contraction extent and duration compared to controls, while the ML-7-treated group does not contract at all post-amputation. In addition, ML-7 supplement immediately after amputation eliminates the contraction period after wounding, even under the background of DPI treatment, indicating downstream regulation of MLCK by ROS. In summary, we revealed that ROS relax wound contraction to allow wound closure through actomyosin regulation, which may offer novel therapeutic strategies for promoting wound closure, especially in chronic wounds.

502S **RAB-10 Antagonizes the AP-1 Clathrin Adaptor to Promote EGFR Signaling in C. elegans** Clare FitzPatrick[1], Christian E Rocheleau[2] [1]McGill, [2]Anatomy and Cell Biology, McGill

Polarized trafficking and signaling is critical to cellular function and development. *C. elegans* Epidermal Growth Factor Receptor (EGFR) localizes to the apical and basal membranes of the vulva precursor cells. However, it is the basal pool of EGFR that is essential for proper vulva induction. We have previously characterized an AGEF-1 (an Arf guanine nucleotide exchange factor), ARF-1 GTPase and AP-1 clathrin adaptor pathway that antagonizes EGFR basal localization and signaling. Loss of *agef-1* results in increased basal EGFR and ectopic vulva induction. We found that the RAB-10 GTPase is required for the mislocalization of EGFR in *agef-1* mutants. Furthermore, RAB-10 is required for the negative effects on EGFR signaling caused by AGEF-1 and ARF-1, but not AP-1, suggesting that RAB-10 functions via AP-1. We found that AP-1 localization in the induced vulva precursor cells is strongly reduced, and this requires EGFR, the downstream Ras GTPase, and RAB-10. Furthermore, RAB-10 localization to cytoplasmic foci is increased in the induced vulva precursor cells and RAB-10 overexpression displaces AP-1 from intracellular compartments into the cytoplasm. We hypothesize that EGFR/Ras signaling promotes EGFR localization to the basal membrane in the vulva precursor cells via activation of RAB-10 which in turn inhibits AP-1. The goal of this study is to determine how EGFR signaling regulates AP-1 localization and RAB-10 activity as well as determine how RAB-10 regulates AP-1.

503S **Securin Regulates the Spatiotemporal Dynamics of Separase** Christopher G.S. Turpin[1], Marian LaForest[2], Dillon Sloan[3], Lindsey Uehlein-Klebanow[4], Diana Mitchell[5], Aaron Severson[6], Joshua N Bembenek[7] [1]Wayne State University, [2]Columbia University, [3]University of North Carolina, [4]Esko Bionics, [5]University of Idaho, [6]Cleveland State University, [7]Mott Center for Human Growth and Development, Wayne State University

Separase is a key regulator of the metaphase to anaphase transition with multiple functions. Separase cleaves cohesin to allow chromosome segregation and localizes to vesicles to promote exocytosis in anaphase. The anaphase promoting complex/cyclosome (APC/C) activates separase by ubiquitinating its inhibitory chaperone, securin, triggering its degradation. How this pathway controls the exocytic function of separase has not been investigated. During prometaphase I in *C. elegans* oocytes, separase and securin colocalize to kinetochore structures at the spindle and in the cortical cytoplasm. Securin is signal decreases substantially over several minutes prior to anaphase onset. After significant securin reduction, separase quickly relocalizes from kinetochore structures to sites of action on chromosomes and vesicles at anaphase onset. Separase does not colocalize with cohesin at the midbivalent region of chromosomes until seconds before cohesin loss and poleward movement of chromosomes. Inactivation of APC/C traps separase on kinetochore structures and prevents separase localization to the midbivalent or vesicles. In contrast, partial securin depletion by RNAi causes precocious separase vesicle localization and abnormal chromosome localization. Given that APC/C and securin depletion have pleiotropic effects, we overexpressed non-degradable Securin fused to GFP (Securin^{DM}) to determine how it affects separase in the presence of normal APC/C activity. Securin^{DM} is stably expressed during anaphase and causes severe embryonic lethality, chromosome segregation defects, polar body extrusion failures and inhibition of cortical granule exocytosis. SecurinDM localizes with separase to kinetochore structures prior to anaphase onset. However, Securin^{DM}interferes with separase localization to vesicles but not the spindle during anaphase. This data suggests that Securin^{DM}inhibits separase binding to vesicle substrates but does not inhibit interactions with kinetochore proteins at the spindle. We conclude that APC/-induced securin degradation controls separase localization. This spatiotemporal regulation will impact the effective local concentration of separase for more precise targeting of substrates in anaphase.

504S **Temperature effects on proteostasis in *C. elegans*** Nhan V.T. Huynh[1], Cole J. Shifferly[1], Jordan B. Carter[1], Grant M. Johnson[1], Erika B. Sorensen[2], Walter R.P. Novak[3] [1]Wabash College, [2]Biology, Wabash College, [3]Chemistry, Wabash College

The 26S proteasome is important for maintaining proteostasis in eukaryotic organisms and possesses multiple proteolytic active sites for cleavage after basic (trypsin-like, TL), acidic (chymotrypsin-like, CT-L) and hydrophobic (caspase-like,CL) residues. The *C. elegans glp-1*(*e2141ts*) mutant grown at restrictive temperature (25 ˚C) has several phenotypes including a germline stem cell proliferation defect, longevity, and a pronounced increase in CT-L proteasomal activity relative to wild type animals and *glp-1*(*e2141ts*) mutants grown at permissive temperature (15 ˚C). Interestingly, different animals show a temperature-dependent reduction in proteasomal activity at higher ambient temperatures. In *C. elegans*, short-term heat stress causes an increase in proteasomal activity in some tissues but no change in others. We set out to assess the proteasomal activity in Glp-1 and wild type animals grown at low (15 ˚C) and high (25 ˚C) temperatures to determine the impact of both temperature and genotype on proteostasis. In alignment with previous studies, we observed that Glp-1 worms have a pronounced increase in CT-L activity when grown at restrictive temperatures, assessed using the fluorogenic substrate Z-GGL-AMC. Proteasome inhibitors such as bortezomib and MG132 are often used to determine background proteasome activity when using fluorescent substrates; however, these inhibitors are not specific for the proteasome and may also inhibit cellular proteases. We observed that while bortezomib inhibits *glp-1*(*e2141ts*) and wild type proteasome activity at 25 ˚C, there was a substantial increase in proteolysis of CT-L substrate in the presence of bortezomib in lysate from worms grown at 15 ˚C, regardless of genotype. To test if additional proteases might be responsible for CT-L cleavage observed in lysate from animals 15 ˚C, we added a protease inhibitor cocktail (PIC). PIC completely abolished the background activity in N2 and *glp-1*(*e2141ts*) animals grown at 15 ˚C. Our results show there are temperature-induced differences in the proteolytic components

responsible for maintaining proteostasis in *C. elegans*. This finding may impact other studies which rely on the assumption that proteostasis is consistent from 15-25 ˚C in wild-type worms.

505S **Molecular and genetic interactions between the DBL-1/BMP signaling pathway and BLMP-1/BLIMP1 regulate organismal traits** M. Farhan Lakdawala[1,2], Matt Crook[3], Tina L. Gumienny[1] [1]Biology, Texas Woman's University, [2]AbbVie (United States), [3]Biology, Wofford College

Bone morphogenetic protein (BMP) signaling helps orchestrate multiple organismal traits throughout the lifetime of animals by regulating target gene expression. Regulation of this signaling pathway is critical for normal development and homeostasis, but an understanding of how this pathway's transcription regulators, called Smads, control target gene expression to generate different traits is not well understood. In the roundworm *Caenorhabditis elegans*, BMP member DBL-1 and its signaling pathway are involved in a spectrum of postembryonic developmental traits. Using the *C. elegans* system, we identified the conserved chromatin remodeler and transcriptional regulator B-lymphocyte-induced maturation protein-1 (BLIMP1) homolog BLMP-1 as a partner in Smad-mediated gene expression regulation. Previous studies in show that BLMP-1 affects a similar array of traits as the DBL-1 signaling pathway. However, the relationship between BMP and BLIMP1 in a post-embryonic developmental context in any animal has not been clarified. While body size was smaller than either single mutant, *blmp-1(tm548)* was epistatic to DBL-1 pathway mutants for lipid content, male tail development, hermaphrodite gonad development, brood size, movement, and survival traits. We showed the DBL-1 signaling pathway and BLMP-1 transcriptionally regulate each other. We also identified a physical interaction between BLMP-1 and the DBL-1 pathway Smads using yeast-two hybrid analyses. Using bioinformatics and qPCR analysis, we showed that Smads and BLMP-1 regulate many common downstream target genes. This work identifies novel interactions between the DBL-1 signaling pathway and BLMP-1, two conserved major transcriptional regulators, that ultimately influence a spectrum of organismal traits.

506S **Regulation of *C. elegans* germline stem and progenitor cell mitosis by developmental and environmental signaling networks.** Eric Cheng, Ran Lu, Abigail Gerhold Biology, McGill University

Equal partitioning of the replicated genome is essential to produce genetically identical daughter cells during mitosis. How mitotic fidelity is affected by physiological changes in developmental or environmental signaling networks remains unclear. *C. elegans* germline stem and progenitor cells (GSPCs) provide an excellent model system in which to investigate the influence of signaling networks on mitotic cells within their native environment. Using *in situ* live-cell imaging, we have shown that caloric restriction and developmental changes affect GSPC proliferation and are associated with a prolonged duration of GSPC mitosis, as measured from nuclear envelope breakdown to anaphase onset. Here we investigated whether developmental signaling pathways also affect GSPC mitotic timing. We find that reducing activity of the insulin receptor *daf-2*/IGFR (*daf-2(e1370*)) leads to an increase in the duration of GSPC mitosis that depends on the mitotic checkpoint. *daf-2(e1370)* also enhances the sterility of checkpoint null mutants and may increase the frequency of chromosome segregation defects, suggesting that reducing *daf-2/*IGFR activity compromises mitotic fidelity in GSPCs. *daf-2(e1370*) mitotic delays depend on the canonical insulin signaling pathway, converging on the regulation of DAF-16/FOXO. However, despite a measurable increase in DAF-16/FOXO nuclear localization in *daf-2(e1370*) GSPCs, we find that this does not predict mitotic duration, and that both DAF-2/IGFR and DAF-16/FOXO are required in the soma, and not in the germline, to affect GSPC mitosis. In addition, although *daf-2(e1370*) phenocopies the effect of caloric restriction on GSPC mitosis, we find that mitotic delays upon caloric restriction require *daf-18*/PTEN and *aak-1/2*/AMPK, but not *daf-16*/FOXO. Overall, our work indicates that distinct signaling networks make genetically separable contributions to the regulation of mitosis in GSPCs and highlight the challenges cells face to preserve mitotic timing and fidelity in complex physiological environments.

507S **A novel molecular mechanism underlying the attachment of myosin A in *C. elegans* striated muscle with the M-line component UNC-89/obscurin** Sarah A Almuhanna[1], Humayra Oishi[2], KarMen Lee[2], Pamela E Hoppe[2] [1]4Clinical Laboratory Sciences, Imam Abdulrahman bin Faisal University, [2]Biological Sciences, Western Michigan University

The goal of this study was to determine the role of different M-line proteins in mediating the attachment of the myosin filaments to the M-line. The contractile unit of striated muscle is the sarcomere, in which myosin-containing thick filaments slide the actin-containing thin filaments to shorten sarcomere length. The M-line is a focal-adhesion-like structure that is anchored at its base to the muscle cell plasma membrane and, through integrin-mediated attachments, to the surrounding basal lamina that is adjacent to the epidermis and cuticle of the body wall. In *C. elegans* the central portion of the thick filament, which attaches to the M-line, is formed by myosin A whereas the filament arms are formed by myosin B. The M-line components that link myosin A to the large structural M-line component UNC- 89/obscurin were investigated using in vivo assays testing the ability of UNC-89/obscurin to associate with myosin A within the contractile apparatus or in ectopic accumulations of filamentous myosin A in different mutant backgrounds. The results showed that the zinc-finger (ZnF) protein UNC-98, but not UNC-82/NUAK kinase or UNC-96, is required for association of UNC-89/obscurin with ectopic myosin

structures. However, UNC-89/obscurin localization to the M-line in the contractile apparatus can occur independently of UNC-98/ZnF. UNC-98/ZnF was detected in the ectopic myosin A structures, but the focal adhesion protein UNC-97/PINCH, which binds to UNC-98/ZnF, was not. Experiments using chimeric myosins revealed that a 170-residue region of the myosin A C-terminus that is known to bind UNC-98/ZnF is sufficient to localize UNC-98/ZnF and UNC-89/obscurin to the M-line in worms lacking endogenous myosin A. An interaction between myosin A and UNC-89/obscurin during development is supported by the highly disrupted localization of UNC-89/obscurin in embryos lacking myosin A. The defects are due to loss of myosin A rather than loss of contraction since UNC-89/obscurin organizes in a mutant lacking the thin-filament protein troponin. Our results identify a novel molecular mechanism underlying attachment of the thick filaments to the M-line and reveal the importance of myosin A in organizing the M-line structure during early development.

508S **Dual transcriptional programs coordinate lipogenic and UPR^{Golgi} programs in *C. elegans* and human cells** Amy K Walker[1], Matthew Fanelli[2], Daniel Higgins[2], Christofer Welsh[2] [1]Program in Molecular Medicine, UMASS Medical School, [2]PMM, UMASS Medical School

Preserving membrane homeostasis is essential for cell structure, organelle function, and signaling. This homeostasis is disrupted when low phosphatidylcholine (PC) causes cells to overproduce stored lipids. We previously discovered that a master regulator of lipogenic transcription, SREBP-1 (*C. elegans* SBP-1), is proteolytically activated in low PC, stimulating fat storage in *C. elegans*, murine liver, and human cell lines. SREBPs are stored in the ER membrane and released by cleavage by Golgi-resident proteases. Subsequently, we found that the phospholipid imbalances activating SBP-1/SREBP-1 are likely to act by disrupting function of the ARF1 GTPase, a key regulator of Golgi/ER traffic. Thus, imbalances in phospholipids can feedback to activate SBP-1/SREBP-1 and lipid production by altering .

In C. elegans, we noted that imbalances in phospholipid levels through interference with PC production drive high-level expression of an ARF1 paralog, *warf-1/arf-1.1*. WARF-1 differs from ARF-1/ARF1 primarily in the N-terminal membrane-insertion helix. We hypothesized that warf-1 might be part of a transcriptional program compensating for the loss of ARF-1/ARF1 function when phospholipid levels are imbalanced. Indeed, we found that *warf-1* is required for *arf-1* RNAi animals to grow past initial larval stages, showing that this paralog biological compensates for the reduction in ARF1 function. Using a screen for transcriptional activators of *warf-1*, we found that *let-607*/CREBH was required for the low-PC activation of warf-1. Interestingly, CREBH proteins reside in the ER and utilize the same protease-dependent activation systems as SREBPs. We hypothesize that blockage of ARF1 activity initiates a bipartite program, stimulating SBP-1/SREBP-1 dependent lipogenesis and activating a protective transcriptional program including a compensatory ARF1 paralog. Finally, we show that *C. elegans* fed E. coli, which lack PC, are sensitized to this stress pathway, however, providing PC-rich *Pseudomonas* strains is protective. Thus, our discovery links membrane-lipid stress responses and ARF1 dysfunction to transcriptional of a compensatory program, providing a novel example of how cells use gene regulatory pathways to sense and respond to changes in lipid homeostasis.

509S **Dosage sensitive screen for maternal factors that effect Cytoplasmic Incompatibility in *Drosophila melanogaster*** Jillian Porter[1], Jillian B. Porter[2], William Sullivan[2] [1]MCDB, University of California Santa Cruz, [2]MCDB, UCSC

Wolbachia is a maternally inherited endosymbiont that manipulates host reproduction to favor infected females. Cytoplasmic Incompatibility (CI) is the most common form of reproductive manipulation. Crosses between infected males and uninfected females result in dramatic reductions in egg hatch (CI) while crosses in which both parents are infected produce normal egg hatch rates (RESCUE). This provides a selective advantage to infected females. The Wolbachia factors that cause CI and Rescue, known as the cif genes, have been identified but their host targets remain unknown. To identify these host targets we have taken advantage of the wealth of genetic reagents in *D. melanogaster* to screen for dosage sensitive enhancers and suppressors of CI. The results of this analysis will be presented.

510S **Decreased muscle performance correlated with increased carbon dioxide exposure in *D. melanogaster* lacking *CG5577*** Abigail Tramell[1], Jennifer Kennell[2] [1]Vassar College, [2]Biology, Vassar College

Many genes in *Drosophila melanogaster* remain uncharacterized, especially genes that have the potential to regulate metabolism, such as the *CG5577* gene. Sequence similarity suggests this gene encodes a likely member of the haloacid dehydrogenase (HAD) domain superfamily of non-protein phosphatases, in particular, a protein similar to human pyridoxal phosphate phosphatase (PDXP). One function of PDXP is to dephosphorylate pyridoxal 5'-phosphate (PLP), the co-enzymatically active form of vitamin B6. PLP is a cofactor for over 140 biochemical pathways, some reactions involving amino acid, neurotransmitter, and glycogen metabolism. Consequences of an increased intracellular concentration of PLP through *PDXP* knockout in mice have been shown to cause muscular and locomotor defects, as well as improved cognition and memory. Our goal for this project is to determine if the *CG5577* gene is the possible ortholog to *PDXP* by knocking out the *CG5577* gene in *Drosophila* and determining if our mutants show similar phenotypes as reported in previous studies. So

far, we have seen that our mutants have decreased flight and climbing performance and an erect wing phenotype compared to our controls. These defects are exacerbated by longer exposure to carbon dioxide prior to testing. In addition, female mutants also showed long-term climbing and flight defects with increasing exposure to carbon dioxide, as well as a fertility defect. These results tell us this gene is involved in muscular ability, fertility, and other aspects of physiology impacted by carbon dioxide exposure. Though the motor defects we see are consistent with previous studies of this possible ortholog in mice, we have also uncovered an interesting interaction between this gene and carbon dioxide exposure that we plan to explore further.

511S **Insights into the role of the acute myeloid leukemia protein, the "LIM-only protein, LMO" in myeloid cells development and function** Sakshi Jain[1], Upendra Nongthomba[1,2] [1]Department of Developmental Biology and Genetics, Indian Institute of Science, [2]Indian Institute of Science

Transcription factors play an important role in maintaining homeostasis during development by controlling the expression of essential genes. However, their aberrant expression due to chromosomal translocations or gene mutation can lead to cancer. LIM-only protein, or LMO, is an important transcriptional regulator of hematopoietic stem cells and progenitor cell development. It regulates erythropoiesis and the survival of T-cell progenitors. Hence, its aberrant expression leads to T-cell acute lymphoblastic leukemia. Additionally, its expression is found to be highest in acute myeloid leukemia and is associated with poor prognosis in these patients. Therefore, this study aims to understand the role of LIM-only protein in myeloid cell lineage specification and function. Our results in *Drosophila melanogaster* have shown that LMO protein, referred to as dLMO or Beadex, is required for plasmatocyte specification, a type of myeloid lineage cell. *Beadex* mutant plasmatocytes have reduced phagocytic ability. The phagocytes of mutant flies feature reduced filopodia length and number, suggesting *Beadex* could function in regulating actin dynamics. *Beadex* has been named a "pathogenesis-specific gene" as its mRNA levels are reduced when infected with a virulent strain of *Pseudomonas aeruginosa.* This study has also shown that *Beadex* mutants are susceptible to infection by Gram-negative bacteria *Salmonella typhimurium*. Moreover, injured flies also die faster, indicating a defective wound-healing process in mutants. Knockdown of *Beadex* orthologs LMO2 and LMO4 in human monocyte-derived THP-1 cells impairs their migration and differentiation potential, respectively. Other LIM domain proteins are known to function as "mechanosensors," which relay information about the mechanical properties of a cell's local environment through force-dependent interactions with the cytoskeleton. LIM proteins directly modulate the dynamics of cytoskeletal networks and act as transcriptional co-activators that alter gene expression. We hypothesize that *Beadex* and other LMO proteins could operate through similar mechanistic principles to modulate phagocytosis in immune cells. Therefore, the present study aims to understand how LIM-only protein engages in regulating the lineage specification in myeloid cells and their role in regulating the kinetics of immune cell movement and function.

512S **The mRNA cap-binding protein eIF4E-Homologous Protein (4EHP) promotes the Integrated Stress Response by stimulating synthesis of NELF-E protein in *Drosophila*** Kristoffer Walsh[1], Christoph Dieterich[2], Hyung Don Ryoo[1] [1]Department of Cell Biology, New York University School of Medicine, [2]Department of Medicine III: Cardiology, Angiology and Pneumology, University Hospital Heidelberg

The Integrated Stress Response (ISR) is a signaling pathway initiated by stress-activated eIF2a kinases that enhance cellular adaptation to various stresses, including amino acid deprivation. Key to ISR-mediated adaptation to amino acid deprivation is the stress-induced synthesis of ATF4, a transcription factor that activates the expression of genes that regulate mRNA translation and enhance amino acid biosynthesis. ATF4-target genes include the translational inhibitor Thor (homolog of mammalian 4E-BP). When active, Thor binds and inhibits the mRNA cap-binding protein eIF4E, a translation initiation factor required for general protein synthesis. How stress-responsive protein synthesis proceeds while eIF4E is inhibited remains an active area of research.

Here, we report that the noncanonical translation initiation factor, eIF4E-Homologous Protein (4EHP), is required for ISR signaling and adaptation to starvation in the *Drosophila* fat body. 4EHP, like eIF4E, is an mRNA cap-binding protein. However, 4EHP is not subject to regulation by Thor. We observe that 4EHP loss results in decreased ATF4 protein and Thor mRNA. Furthermore, loss of 4EHP reduces general amino acid metabolism and enhances vulnerability to nutrient starvation.

Through an RNA-binding screen known as TRIBE, we found NELF-E mRNA as one of the top 4EHP-binding mRNAs. NELF-E encodes one of four subunits that compose NELF, a regulator of RNA polymerase pausing that occurs in many stress-responsive genes. Gene expression profiling indicates that NELF-E knockdown reduces most genes positively regulated by 4EHP, including Thor. Consistently, NELF-E knockdown flies have decreased levels of ATF4 protein and display a nutrient starvation phenotype similar to that seen in loss of 4EHP. Consistent with the idea that 4EHP mediates NELF-E translation, loss of 4EHP reduced NELF-E protein levels with no change in mRNA stability. Together, these findings indicate that 4EHP is a previously unrecognized regulator of ISR signaling that promotes stress adaptation by binding to NELF-E mRNA to mediate its translation. We speculate our observations are consistent with a model by which 4EHP can promote ISR signaling to occur in stressed cells

with Thor-mediated eIF4E inhibition.

513S **A targeted genetic screen for proteins involved in trafficking of neuropeptides in the nervous systems of *Drosophila*** Hardik Bansal, Asha Ali, Tadros a Hana, Veronika G Mousa, Andrew H Michael, Kiel G Ormerod Biology, Middle Tennessee State University

Trafficking of cargoes along the microtubule tracks within the axons of neurons is a critical process necessary for development, maintenance, physiology, and behavioral function. Unlike a typical cell, neurons have axons that can travel long distances, exceeding a meter in humans. Incredible variability exists within the type of cargo and proteins/molecules associated with the cargo vessel. It is well-established that the motor protein kinesin is responsible for anterograde trafficking of axonal cargo, while the dynein/dynactin complex regulates retrograde trafficking. However, there is still much to uncover regarding the various isoforms of these motor proteins as well as the adapter and cargo-associated proteins involved in the precise trafficking dynamics. Here we use a targeted genetic approach using the UAS/Gal4 system combined with RNA interference (RNAi) to knockdown candidate genes and proteins involved in trafficking the organelles synaptic vesicles, mitochondria, and dense core vesicles in motor neurons. Using fluorescently tagged cargo proteins, we conduct live-imaging experiments to quantify changes in trafficking in our RNAi candidate lines. Of the 30 lines we have currently screened, 9 strong trafficking phenotypes were observed when examining cargo localization in the somas, axons, and at the neuromuscular junction. We are currently looking for changes in NMJ structure using immunostaining for pre-and post-synaptic proteins as well as performing electrophysiological recordings to examine changes in synaptic efficacy. We are additionally looking for changes in larval crawling behavior associated with these strong deficits. Taken together, we are uncovering novel roles for motor proteins, adapter and cargo-associated proteins involved in axonal trafficking of organelles, and assessing how deficits are associated with the downstream function of motor neurons.

514S **Examining the role of evolutionary changes in *Esterase 6* protein function in *Drosophila sechellia* toxin resistance** Idenya Bala-Mehta Biology, Wesleyan University

Drosophila sechellia is an island endemic host-specialist fruit fly native to the Seychelles Islands. Central to specialization on the fruit of *Morinda citrifolia* was the evolution of resistance to the defense compounds made to deter herbivory. The primary toxins involved are volatile medium chain fatty acids and fatty acid esters. Prior work from our group showed that *Esterase 6* (*Est6*) plays a role in resistance to the toxic effects of *M. citrifolia* volatiles. Both changes in gene expression and protein function are hypothesized to have contributed to evolutionary differences in *D. sechellia Est6* function. Here we begin to investigate how species-specific differences in *Est6* sequence, including a non-conservative substitution that projects into the enzyme's active site, are involved in catalysis. Using a published high-resolution crystal structure of *Est6* we used computational binding simulations generated by multiple different software platforms to test hypotheses about structural and predicted functional differences associated with known protein sequence differences. This work was then followed by *in vitro* analysis of the different protein variants tested with different *M. citrifolia* derived substrates. This method has thus far suggested that previously overlooked volatiles are important components of *M. citrifolia* toxicity and evolved *D. sechellia* resistance to them is mediated by derived *Est6* protein function.

515S **Sorting of Neuropeptides into Dense Core Vesicles.** Veronika g Mousa, Hardik Bansal, Kiel G Ormerod Biology, Middle Tennessee State University

Neuromodulation through the release of neuropeptides stored within dense core vesicles (DCVs) plays an essential role in regulating behavior. Given the diversity of neuropeptides encoded in animal genomes and their distinct effects on behavior, it is critical to define how different neuropeptides are sorted, packaged, and released. Here, we use fluorescent-tagging of multiple independent neuropeptides in *Drosophila* motor neurons to follow their sorting and transport. We find unique neuropeptides co-expressed within the same neuron largely sort into separate DCVs that do not intermix during their lifecycle. In addition, the same neuropeptide encoded from different mRNAs also sort to unique DCV populations, while multiple neuropeptides encoded in the same mRNA transcript are usually sorted, packaged, and transported together. Altering the expression of key enzymes involved in neuropeptide processing did not disrupt their sorting or transport, but caused an accumulation of DCVs at synapses, providing a link between neuropeptide processing and their subsequent release. These data indicate cargo of single DCVs can be associated with neuropeptides generated from one mRNA population that do not intermix during biogenesis or transport, allowing DCV release to activate specific downstream signaling pathways associated with their individual neuropeptide content.

516S **Interweaving Autophagy and Exosome Functions: Unraveling CryAB** Ziwei Zhao, David Brooks, Yungui Guo, Erika Geisbrecht Kansas State University

Phosphorylation reactions performed by protein kinases are one of the most studied post-translational modifications

within cells. Much is understood about conserved residues within protein kinase domains that perform catalysis of the phosphotransfer reaction, yet the identity of the target substrates and downstream biological effects vary widely among cells, tissues, and organisms. Here we characterize key residues essential for NUAK kinase activity in *Drosophila melanogaster* myogenesis and homeostasis. Creation of a *NUAK* kinase-dead mutation using CRISPR/Cas9 results in lethality at the embryo to larval transition, while loss of NUAK catalytic function later in development produces aggregation of the chaperone protein CryAB in muscle tissue. Yeast two-hybrid assays demonstrate a physical interaction between NUAK and CryAB. We further show that a phosphomimetic version of NUAK promotes the phosphorylation of CryAB and this post-translational modification occurs at two previously unidentified phosphosites that are conserved in the primary sequence of human CryAB. Mutation of these serine residues in *D. melanogaster* NUAK abolishes CryAB phosphorylation, thus proving their necessity at the biochemical level. Numerous human CryAB mutations cause cataracts, myofibrillar myopathy and/or cardiomyopathies. Modeling four of these human mutations in *D. melanogaster* muscle tissue causes varying degrees of muscle degeneration and the abnormal accumulation of CryAB, ubiquitin, and p62 aggregates. Interestingly, the small GTPase Rab 27 also colocalizes with a proportion of the CryAB-positive structures. This suggests that CryAB mutations not only affect the degradation of intracellular misfolded proteins but also alter the degradation and exocytosis of multivesicular bodies (MVBs). Further analysis reveals phenotypic clustering of aggregate morphologies that correlate with the location of the human mutations within the CryAB protein structure. These studies together highlight the importance of kinase activity regulation and provide a platform to understand the molecular role of CryAB in normal and diseased states.

517S **Developmental periodic oscillations of JAK/STAT signaling serve as a switch for maturation-inducing steroid pulse by regulating lipid droplet pool.** Jie Sun, Calder Ellsworth, Adam Aldahir, YICHUN HUANG, Wu-Min Deng Tulane University School of Medicine

The onset of sexual development is closely linked to changes in hormonal secretory activities of the pituitary gland in vertebrates and the steroidogenic prothoracic gland (PG) in insects. Steroid pulse frequency, amplitude, and duration are crucial for triggering insect sexual maturation through initiating genetic response programs. Here we reveal that the oscillations of JAK/STAT signaling, regulating the periodic dynamics of lipid droplet pool, contribute to the initiate sexual maturation. Lipid droplets, serving as storage organelles, maintain lipid and steroids homeostasis, dynamically facilitating coordination and communication between different organelles. The rhythmic oscillations of JAK/STAT signaling control a bidirectional loop of lipid droplet biosynthesis and degradation in PG cells. In late third-instar larvae, accompanied by the release of abundant steroids from PG, intracellular JAK/STAT signaling is significantly suppressed, thereby inhibiting lipolysis and promoting lipogenesis. If external inductions such as hyperinflammation or wounds activate JAK/STAT signaling during this period, it reverses lipid metabolism, leading to rapid degradation of lipid droplets and abnormal cholesterol accumulation. Ultimately, this hinders steroid hormone biosynthesis, preventing larvae from entering the pupal stage. We further found that the BTB zinc-finger transcription factor *fruitless (fru)* is a key participant in regulating lipid metabolism and negatively regulates the lipid droplet accumulation in PG cells by responding to JAK/STAT signaling levels in the body. Specifically, we show that Fru dynamic expression within the PG cells: a high concentration of Fru in the nuclei is concomitant with low-titer ecdysone levels in the body, and the protein is absent from PG nuclei at developmental stages when high-titer ecdysone pulses occur. Depletion of Fru in the PG accelerates larval development by causing precocious ecdysone signaling and a failure to repress ecdysone pulses. In contrast, excessive Fru expression in the PG arrests development that can be rescued by administrating an active Ecdysone. In summary, our findings elucidate how the JAK/STAT signaling pathway controls the decision-making process of maturation by maintaining a delicate balance in lipid metabolism and steroid homeostasis.

518S **An EMS screen for neuropeptide biology in the nervous system of *Drosophila*** Amber Washington, Veronika g Mousa, Asha Ali, Marina Khalil, Kiel G Ormerod Biology, Middle Tennessee State University

Neuromodulatory substances regulate critical processes spanning from regulated secretion to physiology and behaviour. It is therefore not surprising that most genomes encode hundreds of neuromodulatory substances and their receptors. The *Drosophila* genome encodes over 30 genes for neuropeptides alone. Neuromodulatory substances like neuropeptides are packaged within cells in large electron dense structures known as dense core vesicles (DCVs). DCVs are responsible for the transport, storage, and release of proteins and neuropeptides at multiple cellular locations and are known to be involved in a multitude of biological processes including synaptogenesis, synaptic transmission, synaptic plasticity, and others. However, much of cellular machinery involved in sorting, processing, trafficking, and ultimately secretion of DCV contents remains largely unknown. Here we have taken advantage of the genetic and molecular toolkit of *Drosophila* to conduct a genetic screen for changes in cellular localization of fluorescently labelled neuropeptides. We have identified several critical proteins necessary for proper processing of prepropeptides into bioactive neuropeptides ultimately impacting their ability to be trafficked to and undergo regulated secretion at the neuromuscular junction. Using this EMS screen and other approaches in our lab, we are beginning to characterize cellular mechanisms of sorting of different uniquely tagged DCV cargo. Lastly, by employing quantal resolution imaging of vesicle fusion at individual active zones, we are also characterizing the synaptic

machinery mediating trafficking and secretion of DCVs. The novel mutants isolated here enable a more comprehensive understanding of the critical mechanisms of neuropeptide sorting, trafficking, and secretion *in vivo*.

519S **TGFβ/Activin signaling positively regulates mitochondrial genome maintenance and glycogen homeostasis** Heidi Bretscher, Michael O›Connor University of Minnesota- Twin Cities

Mitochondria are one of the central regulators of metabolic homeostasis providing a significant source of ATP and metabolic intermediates required to sustain organismal growth and survival. Unlike most organelles, mitochondria contain their own genome and transcriptional machinery. The protein complexes required for mitochondrial oxidative phosphorylation and ATP production are composed of both nuclearly and mitochondrialy encoded subunits. Thus the balance of nuclearly and mitochondrialy encoded subunits must be maintained for optimal function. We have uncovered a new role for the TGFβ/Activin ligand Actβ in regulation of mitochondrial DNA maintenance. Loss of Actβ results in decreased mitochondrial DNA and decreased expression of nuclearly encoded mitochondrial transcriptional machinery including the mitochondrial RNA polymerase, *polrMT*, as well as two mitochondrial DNA helicases, *twinkle* and *suv3*. Accordingly, we see down regulation of mitochondrialy encoded, but not nuclearly encoded, subunits required for oxidative phosphorylation. As a readout for mitochondrial function, we measured mitochondrial membrane potential and ATP levels in the muscle of control and *actβ* mutant larvae. We found that *actβ* mutants have increased mitochondrial membrane potential, but decreased ATP levels suggesting that Actβ is required for efficient oxidative phosphorylation. In order to understand the metabolic consequences of decreased mitochondrial DNA in *actβ* mutants we measured glycogen and lipid levels and found that Actβ is a strong positive regulator of glycogen storage, with mutants having only about one third the glycogen of control animals. Importantly, knockdown of either *mtRNApol/PolrMT* or *mtDNA helicase/twinkle* in muscle led to decreased organismal glycogen levels establishing a direct link between mitochondrial genome maintenance and glycogen homeostasis. Thus, our model is that Actβ regulates glycogen homeostasis via expression of nuclearly encoded mitochondrial transcriptional machinery required for mitochondrial genome maintenance.

520S **Investigating the interaction between Uif, Mmp1 and PNPase with insulin signaling in tissue-specific growth of the larval trachea** Zihao Yu, Robert Ward Case Western Reserve University

Most animal species show allometric growth, which means that different organs and tissues grow at different rates relative to each other. Understanding the mechanisms of how different tissues grow is important, since it can provide insight about unique signals and pathways required for development of different organs. The Drosophila larval trachea is an excellent model since they show tissue-specific growth and the trachea can be easily visualized due to optical properties of the air-liquid interface in the trachea. In addition, we and other labs have identified genes required for growth specifically in the larval trachea, including *uninflatable (uif)* and *matrix metalloproteinase 1 (mmp1)*, which are positive regulators of tracheal growth and *Polynucleotide phosphorylase (PNPase)*, which is a negative regulator of tracheal growth. We have also identified a mutation in an uncharacterized gene that is also a negative regulator of *growth (l(3)12265).*
The larval trachea is an endoreplicating tissue (ERT) and previous work from the Edgar lab showed that cellular growth in ERTs are regulated through the insulin signaling pathway. Tracheal-specific expression of dominant active, dominant negative and RNAi constructs to insulin signaling components results in predictable alterations in tracheal growth. We therefore hypothesize that these tracheal specific growth regulators act to modulate insulin signaling specifically in the larval trachea.
Here, our goal is to investigate how tracheal growth genes interact with the insulin signaling pathway to regulate growth. We will measure readouts of insulin signaling in *uif, Mmp1, PNPase and l(3)12265* mutant larvae including phospho S6 kinase and the expression and localization of tGPH. We will also perform genetic experiments to manipulate insulin signaling in these mutants and test for enhancement or suppression of the tracheal growth phenotypes.

521S **FoxO is necessary for heat-induced fat loss.** Jin Seo, Tucker Hopkins Biology, Rogers State University

Animals must develop mechanisms to cope with environmental challenges for their survival. Daily and seasonal fluctuating temperatures are inevitable and common obstacles, imposing pressure to adapt to heat in all living organisms. Organisms tend to lose their stored body fat at temperatures higher than their optimum, which is observed in mammals as well as insects. However, the mechanisms controlling this heat-induced fat loss have not been fully understood. We hypothesized that organisms must have developed a specific mechanism, likely ancient and common throughout all animal kingdoms, as temperature fluctuations are a persistent occurrence. When fruit flies are exposed to heat, they significantly lose their body fat. Analysis of gene expression in the flies incubated at an elevated ambient temperature revealed significant changes in genes regulating the insulin singling pathway as well as lipid metabolism. The expression of various insulin like peptides (Ilps) was differentially regulated. Further, insulin signaling was suppressed in *Drosophila* S2R+ cells at an elevated temperature without Ilp regulation, indicating that heat autonomously inhibits the insulin signaling pathway regardless of Ilps. *In vivo* expression of constitutive active form of insulin receptor completely blocked heat-induced fat loss. Subsequently, we

demonstrated the insulin receptor- FoxO branch in the insulin signaling pathway is necessary for heat-induced fat loss.

522S **X-ray Radiation Induced Cellular Plasticity in the Drosophila Wing Imaginal Disc** Michael Shiferaw Molecular, Cellular & Developmental Biology, University of Colorado, Boulder

Ionizing radiation, while a potent weapon against cancer, appears to induce a unique adaptability in cells, enabling them to survive and relocate within tissues. This phenomenon is distinctly observed in the Drosophila wing imaginal disc where post-radiation, hinge cells migrate unidirectionally to damaged pouch areas, undergoing transformation to replace lost or damaged cells. Such cell fate changes and migrations prompt exploration into the foundational intercellular communications driving this adaptability and plasticity. To tackle this, the study aims to decipher the intercellular signaling orchestrating this migration, with a spotlight on the pivotal ligand-receptor interactions. Through an in-depth analysis of a comprehensive RNASeq dataset (Ledru et al., 2022), complemented by validation techniques such as in-situ hybridization, we intend to identify the key players and underlying mechanisms. Emphasizing ligand-receptor interactions is motivated by their central role in signaling cascades, with the potential to trigger downstream migration-driving events. We will utilize pouch and hinge specific drivers to overexpress or knockdown candidate genes, allowing us to further explore the roles of these genes in regulating cellular plasticity and migration. By delineating these radiation-induced cellular behaviors, our goal is to deepen the understanding of cellular plasticity in response to radiation, subtly alluding to its wider implications in refining therapeutic approaches.

523S **Local nuclear to cytoplasmic ratio regulates chaperone-dependent H3 variant incorporation during zygotic genome activation** Anhsua Bhatt[1], Madeleine Brown[1], Aurora Wackford[2], Yuki Shindo[2], Amanda A Amodeo[2] [1]Biology, Dartmouth College, [2]Dartmouth College

The process of differentiation is characterized by lineage-specific gene expression and chromatin micro-environments. Cells in the early embryo of many species have relatively unstructured chromatin that lacks active and inactive domains typical of more differentiated cells. In many species, including Drosophila, these regulatory domains are established during zygotic genome activation (ZGA). In Drosophila, ZGA occurs after 13 fast, reductive, syncytial nuclear divisions during which the nuclear to cytoplasmic (N/C) ratio grows exponentially without cytoplasmic growth. These divisions incorporate maternally loaded cytoplasmic pools of histones, into the chromatin. Previous work has shown that the relative amount of replication-coupled histone H3 decreases while its variant H3.3 increases on chromatin in the cell cycles leading up to ZGA. H3.3 is known to associate with active promoters and enhancers as well as heterochromatin domains, suggesting a link between H3.3 incorporation and chromatin changes during ZGA. Here, we examine how H3.3 incorporation is regulated during ZGA. We identify a decrease in the overall nuclear import of H3, but not H3.3, over the final pre-ZGA cycles. We also observe an N/C ratio-dependent increase in H3.3 incorporation at high N/C ratios within mutant embryos with uneven N/C ratios. We evaluate the contributions of chaperone binding and gene expression by creating H3/H3.3 chimeric proteins at the H3.3A locus. We measured H3-like incorporation of those chimeras with the H3 chaperone-binding site. We test the specificity of the H3.3 chaperone pathways for H3.3 incorporation using Hira (H3.3 chaperone) mutant embryos. Overall, we propose a model in which local N/C ratio and chaperone binding regulate differential incorporation of H3.3 during ZGA.

524S **Atypical FGF ligand Pyramus, a Type I transmembrane protein, undergoes intracellular cleavage that affects mesodermal cell fate** Chen Zhang, Jingjing Sun, Vincent Stepanik, Angelike Stathopoulos Biology and Biological Engineering, California Institute of Technology

Fibroblast Growth Factor (FGF) signaling is an evolutionarily conserved receptor-ligand signaling pathway that contributes to multiple vital developmental functions including cell proliferation, differentiation, and migration. During heart development, the fibroblast growth factor (FGF) Pyramus (Pyr) and its receptor (FGFR) Heartless (Htl) play a vital role in the differentiation of pericardial precursors through the activation of *even-skipped (eve)* gene expression via the mitogen-activated protein kinase (MAPK) pathway. In both *htl* and *pyr* mutants, a complete loss of *eve* expression and, subsequently, failure of pericardial cell differentiation and heart formation are observed.While most FGF signaling ligands are secreted in entirety, our previous study identified Pyr as the first characterized transmembrane FGF protein (Stepanik, Sun et al., *Curr Biol* 2020). Its transmembrane domain (TMD) separates an extracellular FGF-homologous portion from an extended intracellular domain (ICD) of unknown function. We showed that cleavage of Pyr results in various truncated forms of Pyr that lack the TMD and/or the extended ICD. These isoforms are associated with different levels of diphosphorylated form of ERK (dpERK), which indicates that different Pyr truncations have varying capacity to activate MAPK signaling. In this new study, we investigated Pyr cleavage dynamics in vivo utilizing a new sensor, a Pyr fusion protein in which GAL4 is inserted in the intracellular domain (Pyr-GAL4-ICD) that monitors ICD detachment from the TMD. By monitoring Pyr-GAL4-ICD driven expression of GFP relative to eve expression, we show that cleavage of Pyr takes place as early as stage 9 of embryogenesis and is coincident with eve expression in Eve pericardial cells (EPCs). Furthermore, *pyr* truncation mutants were further examined finding that deletion of the Pyr ICD is associated with change in *eve* and *runt* expression dynamics. Our results point to a role for Pyr cleavage in supporting temporal

regulation of MAPK signaling. Mammalian FGF proteins FGF21 and FGF23 also interact differently with their FGFRs depending on their cleavage states. By understanding the molecular mechanisms controlling Pyr cleavage and the effects on downstream intracellular signaling, we may elucidate novel regulatory mechanisms relating to the FGF signaling pathway including the possibility that ligands also signal in reverse.

525S **The Role of Myosin in Pupal Wing Expansion** Anni Yi[1], Kenneth D Irvine[2] [1]Molecular Biology and Biochemistry, Rutgers University New Brunswick, [2]Molecular Biology and Biochemistry, Waksman Institute of Microbiology

During the pupal stage of Drosophila melanogaster, it is known that the pupal wing flattens and expands to form the adult wing and that pupal wing cells change shape and flatten during this expansion. However, this crucial step in pupal development is still not very well understood in its early stages. We are investigating the behavior of the protein non-muscle Myosin II, encoded by the gene Spaghetti Squash in Drosophila, during pupal wing expansion since it is well known to have structural functions within the cell such as helping to facilitate cell migration and cell division. We investigated Myosin II's localization in fixed pupal wings of various ages by performing in vivo experiments using fluorescent protein-tagged Myosin II transgenes. While previous studies have shown that Myosin II relocalizing to the lateral sides of the cell from 6-8 hours post pupariation plays a role in pupal wing expansion, our results suggest that it may be a different mechanism that controls the cell shape changes involved in pupal wing expansion. Additionally, we have found the presence of previously unreported bright, strongly apical localizing Myosin II structures towards the end of this 2-hour window in pupal wing development. Currently, we are trying to understand the role that these structures play in pupal wing development using ex vivo experiments, which could provide new insight into the biomechanical controls of the early stages of pupal wing development.

526S **The role of ubiquitin and multivesicular body formation in the stem cell niche of the *Drosophila melanogaster* testis** Sheetal Kooduvalli, Mara Grace, Erika Matunis Johns Hopkins University

Post-translational protein modifications such as ubiquitylation control protein interactions, stability, function, and localization, all of which are essential for maintaining optimal conditions in the cell. The *Drosophila melanogaster* testis stem cell niche serves as an ideal model to understand the role of ubiquitylation in stem cell maintenance. The niche supports two different population of cells- germline stem cells (GSCs) which later differentiate into sperm and cyst stem cells (CySCs) which support the germline. Here we find that RNAi-mediated knockdown of the endosomal sorting complexes required for transport (ESCRTs) in CySCs results in a dramatically enlarged niche due to an increased number of cells, a striking phenotype, as niche cells are normally quiescent. ESCRTs recognize ubiquitylated receptors in endosomes and control downstream formation of multivesicular bodies (MVBs) necessary to silence receptor signaling, leading us to a model where ESCRTs mediate signaling from CySCs to their niche. Consistent with disruption of endocytic trafficking and MVB formation we detected ubiquitin and early endosome accumulation in CySCs following ESCRT knockdown. We hypothesize that ubiquitylated and internalized receptors accumulate in endocytic vesicles and continue to signal to the niche, driving hub cell proliferation. Disruption in ESCRT functioning is seen in many diseases such as cancer and we hope to understand the etiology through pathways that lead to abnormal cellular communication.

527S **Large secretory vesicles employ a unique mechanism of exocytosis** Eyal Schejter[1], Kumari Kamalesh[1,2], Tom Biton[1,2], Nadav Scher[2], Shari Carmon[1], Yael Elbaz-Alon[2], Ori Avinoam[2], Benny Shilo[1] [1]Molecular Genetics, Weizmann Inst Sci, [2]Biomolecular Sciences, Weizmann Inst Sci

Secretion of adhesive ("glue") glycoproteins from the apical surface of *Drosophila* larval salivary gland cells to the gland lumen, serves as a prominent model for exocytosis via exceptionally large vesicles. This process poses significant challenges to various aspects of the secretory apparatus, including the mechanisms of vesicle fusion, cargo release and cell membrane homeostasis. Our studies address the unique cellular and molecular mechanisms underlying key junctures along this specialized route of secretion. We find that the fusion pore, through which vesicle content is released, displays a dynamic behavior, involving expansion, stabilization and eventual contraction. Progression through these steps is regulated by Bin-Amphiphysin-Rvs homology (BAR) domain proteins, most notably the fly homolog of the I-BAR domain protein MIM. Following fusion, a Rho GTPase-based pathway, employing balanced and regulated input from specific RhoGEF and RhoGAP elements, is activated, leading to coating of the vesicles by a contractile actomyosin network, which is constructed in a temporally biphasic manner. Contraction of this network serves multiple roles: content release to the lumen, regulation of fusion pore dynamics, and notably, "crumpling" of the vesicle membrane, which keeps it insulated from the apical cell membrane, thereby maintaining membrane homeostasis.

528S **Role and Regulation of Polarity Proteins during Mitosis** Sarah JL Robinson, Gayaanan Jeyanathan, Milena Pellikka, Ulrich Tepass University of Toronto

In cancer, the shift from a hyperplastic to a neoplastic tumor capable of metastasis is partly due to loss of cell polarity. Our

recent work in Drosophila embryonic epithelia has established that morphogenetic movements including mitosis challenge epithelial polarity, thus threatening tissue integrity. Reducing morphogenesis by, for instance, blocking cell division or cell ingression, releases pressure from the polarity machinery and ameliorates polarity protein loss-of-function mutant phenotypes. This interaction is dose-dependent; the more morphogenetic activity, the more polarity is required to maintain tissue integrity. Cell division poses a unique challenge to epithelial polarity, as apical-basal polarity is transiently lost during division, a process called 'mitotic polarity oscillation'. We and others have found that apical polarity proteins and adherens junction components are removed from the apical membrane at the onset of mitosis and return after the cell divides. Additionally, the apical polarity protein Crumbs (Crb) is crucial for re-establishing and maintaining polarity in dividing tissue. However, the role of Crb relocalization during division is not yet understood.

To further expand on the importance of polarity in morphogenetically active tissue, we investigated other polarity proteins, and whether their loss-of-function phenotypes can be rescued by removing morphogenetic movement. We focus on basolateral polarity proteins Scribbled, Discs Large, Lethal Giant Larvae, and Yurt. We either blocked cell division or cell ingression, or both movements simultaneously in a basolateral polarity protein loss-of-function background. We will present our findings and compare them to interactions between morphogenesis and apical polarity proteins. We are also interested in understanding mitotic polarity oscillation, and whether the relocalization of apical Crb is a necessary step for proper mitosis or a by-product of cell division. We hypothesize that Crb removal facilitates formation of a uniform actin cytocortex which aids in mitotic rounding prior to division. Using a Crb protein lacking a target site for Clathrin-mediated endocytosis (CrbRR), we aimed to prevent the loss of Crb from the apical membrane and observe any changes in mitosis. Preliminary findings suggest that CrbRR is removed from the membrane of mitotic cells in contrast to interphase cells, suggesting that apical plasma membrane removal does not engage a Clathrin-dependent mechanism.

529S **Developing Single-Cell Mass Spectrometry Methods to Identify Candidate Actin Mesh Regulatory Proteins** Merin M Rixen, Joseph Loo, Margot E Quinlan Chemistry and Biochemistry, UCLA

During egg development, oogenesis, actin filaments form a meshwork inside of the oocyte which aids in establishing oocyte polarity. Polarity establishment is a critical event that determines the major body axes of the egg and ensures the development of healthy offspring. This is a highly conserved process and analogous actin mesh networks have been observed in various species, including fruit fly (*Drosophila melanogaster*), worm, mouse, and human oocytes. The *Drosophila* actin mesh fills the oocyte during mid-oogenesis but disappears at the onset of late oogenesis. This timely removal is integral for the proper establishment of germline polarity. If the mesh is removed too early or too late, oocyte polarity is not established or has delayed establishment, and the fertility of the fly is decreased. However, little is understood about the mechanism behind the mesh removal. This research aims to identify candidate proteins that regulate actin mesh removal and investigate their functions in relation to mesh disappearance. I will use single-cell mass spectrometry to measure protein levels and abundance changes in single *Drosophila* oocytes between mid and late egg development stages. This technique will allow me to identify the proteins that reflect relevant abundance changes between the stages of mesh maintenance and disappearance. I will complement my mass spectrometry analysis by using fly knockdown lines to determine what role these proteins have on actin mesh disassembly. Identifying the proteins that regulate actin mesh removal, and determining their roles, will advance our comprehension of the mechanisms and machinery that direct actin mesh regulation. Our findings will promote a deeper understanding of the developmental defects that arise from improper egg maturation across multiple species. Furthermore, the relevant results from this research may be extended to other systems of organized actin assembly that govern important cellular processes.

530S **Disrupted endosomal trafficking of polarity proteins causes congenital trachea-esophageal separation anomalies** Nicole Edwards[1], Adhish Kashyap[2], Alissa Warren[2], Zachary Agricola[2], Alan Kenny[2], Yufeng Shen[3], Wendy Chung[3,4], Aaron Zorn[2] [1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, [2]Cincinnati Children's Hospital Medical Center, [3]Columbia University Medical Center, [4]Harvard Medical School

Trachea-esophageal defects are life-threatening congenital anomalies that arise when the trachea and esophagus do not correctly separate from the common anterior foregut tube. The genetic causes of trachea-esophageal anomalies are not well known, and how the trachea and esophagus normally separate during embryonic development is not well understood. In a collaborative network called the CLEAR Consortium, we are using animal models and patient genome sequencing to discover new trachea-esophageal anomaly-related variants and determine their disease mechanisms. Using the complementary advantages of mouse and *Xenopus* embryos, we recently defined a novel endosome-mediated epithelial remodeling process that is critical for separation of the foregut into tracheal and esophageal tubes. We aimed to determine the cellular mechanisms regulated by endosomal trafficking by mutating the key endosomal pathway proteins Dynamin, Rab5a, and Rab11a in *Xenopus*. We found that disrupting endosome trafficking leads to trachea-esophageal fistulas, occluded trachea-esophageal lumens, and epithelial cell disorganization in the transient septum connecting the trachea and esophagus. We also

find significantly altered localization of the polarity proteins laminin and Vangl2 in endosomal mutant embryos, suggesting that these proteins may be trafficked by recycling endosomes to maintain epithelial apical-basal cell polarity during trachea-esophageal separation. We also observed disrupted polarity in *Xenopus* mutants of novel patient variants in genes predicted to function in endocytosis, suggesting that disrupted endosome-mediated polarity maintenance may be a common disease mechanism underlying human trachea-esophageal anomalies. This work is funded by NICHD P01 HD093363.

531S **Modeling single-cell phenotypes in wild-type and regulatory mutant yeast to explore growth control and stress defense** Rachel A Kocik[1,1,2], Andrew C Bergen[1,2], James Hose[1,2], Megan McClean[2,3,4], Audrey P Gasch[1,2,3] [1]Genetics, University of Wisconsin-Madison, [2]Center for Genomic Science Innovation, University of Wisconsin-Madison, [3]University of Wisconsin Carbone Cancer Center, University of Wisconsin School of Medicine and Public Health, [4]Biomedical Engineering, University of Wisconsin-Madison

Cells must respond dynamically to a multitude of environmental changes to maintain cellular homeostasis and protect against environmental stresses. To properly manage changing environments, cells utilize signaling pathways; however, the ways in which these pathways are controlled, including protein activation, localization dynamics, and gene expression are not fully understood. In the case of environmental stresses, many studies done in bulk culture fail to capture cellular heterogeneity, which can reveal unique phenotypic relationships found only in subsets of cells. To investigate what gives rise to variation in stress response, we paired live-cell imaging with a microfluidics system to study multiple phenotypes of single *Saccharomyces cerevisiae* cells before and after salt stress. We simultaneously followed cell and colony growth, cell size and volume, cell-cycle phase, and nuclear localization of two Protein Kinase A (PKA) co-regulated stress responsive transcription factors, transcriptional activator Msn2 that regulates stress-induced genes and repressor Dot6 that represses expression of ribosome-biogenesis genes. We found unexpected differences in Msn2 and Dot6 localization changes across cells, revealing unanticipated discordance in their regulation. This discordance persists even in cells lacking a PKA regulator, phosphodiesterase Pde2; however, the overall distribution of both Msn2 and Dot6 nuclear localization was significantly lower in cells lacking Pde2. Interestingly, post-stress growth recovery was positively correlated with activation of the Dot6 repressor. In contrast, cells lacking Dot6 (and paralog Tod6) displayed slower growth acclimation even though they grow normally in the absence of stress. Cells lacking Pde2 also displayed slower post-stress growth, further underscoring the role of Dot6 activation in the stress response. Unlike cells lacking Dot6, cells without Msn2 (and paralog Msn4), exhibited faster post-stress growth, despite growing normally without stress. While Msn2 and Msn4 are not required for basal stress tolerance, they are needed for cells to survive a secondary stress, suggesting Msn2 activation may be predictive of cells acclimation to a secondary stress. Our results demonstrate that, while cells' responses are highly dynamic, specific cellular attributes can partially predict how cells will respond to stress.

532S **Characterization of the developmentally important disaggregation activities of Abcf proteins** Sydney Skuodas, Amy Clemons, Bryan Phillips, Jan Fassler University of Iowa

The *ABCF* gene family encodes ABC type ATPases. In yeast, members of this family are involved in translation and ribosome biogenesis, however, this gene family is related to the fungal specific New1 protein, which exhibits Hsp104-independent disaggregation activity. Analyses of potential disaggregation activity in yeast revealed that depletion of Abcf proteins in yeast reduces the rate of resolubilization of denatured proteins and reduces the aggregate burden of cells carrying an Htt-Q97 reporter.

Animal Abcf proteins complement the yeast defects and knock-down in *Xenopus* embryos result in developmental defects. In *C. elegans* we find that ABCF-1 limits amyloid aggregation and is required for maternal fecundity and embryonic survival. Some aggregates that accumulate in ABCF-1 deficient animals contain the RNA binding protein CAR-1, which is normally localized to RNA processing bodies. These data suggest that aggregation-prone RNA processing membraneless organelles are regulated by ABCF-1.

Yeast Arb1 (Abcf2) co-localizes with heat-denatured aggregates suggesting a possible direct interaction with aggregate clients. However, Arb1 does not co-localize with heterologous amyloid reporters. Because endogenous amyloids (prions) significantly influence heterologous reporter aggregates, we sought to investigate the role of Arb1 in endogenous amyloid processing. Our results show that the Arb1 protein co-localizes with the Sup35 encoded [PSI$^+$] prion and that *ARB1* overexpression alters the number and spectrum of [PSI$^+$] aggregates while the absence of *ARB1* influences the aggregate load in strains carrying the weak [PSI$^+$] prion variant. These results are consistent with a direct role for Arb1 in disaggregation as predicted by its phylogenetic relationship with the yeast disaggregase, New1.

We have also begun to investigate possible Arb1 co-factors. A functional interaction with Hsp104 is suggested by our observation that the two proteins co-localize following heat shock and that *ARB1* overexpression can suppress the thermosensitive phenotype of *hsp104* mutants. We further find that *ARB1* overexpression lysates have a large impact on the

extent to which denatured luciferase can be reactivated in the presence of low levels of recombinant Hsp104. We propose that Hsp104 and Arb1 may work sequentially or collaboratively in refolding disordered aggregates. Additional chaperone mutant lysates are being screened for changes in Arb1 and Hsp104 mediated refolding.

533S **Modulatory inputs into the MAPK pathway that controls filamentous growth in yeast** Atindra N Pujari, Paul J Cullen Biological Sciences, State University of New York at Buffalo

Signal transduction pathways control the execution of cellular responses to intrinsic and extrinsic stimuli. Erroneous activation of signaling pathways and their cognate transcription factors due to gain-of-function and loss-of-function mutations in genes encoding positive regulators and negative regulators, respectively, have been linked to diseases such as cancer in humans. A type of signaling pathways that are highly conserved are mitogen-activated protein kinase (MAPK) pathways. Model organisms like yeast contain MAPK pathways and serve as a platform to learn how these pathways are regulated. To better understand the regulation of MAPK pathways, a genetic screen was performed by measuring the activity of a MAPK pathway reporter combined with next-generation sequencing. Mutants were identified that showed elevated activity of a crosstalk growth reporter (*FUS1-HIS3*) whose activity provides a readout of the MAPK pathway that controls filamentous growth. Spontaneous mutants were selected on media lacking histidine and containing 3-amino-triazole, a competitive inhibitor of the His3p enzyme. In total, 159 mutants were identified and analyzed by secondary screens for invasive growth by the plate-washing assay, filament formation by microscopy, and measurement of pathway activity by the β-galactosidase assay. The genomes of thirty-two separate mutants showing strong reporter activity were individually sequenced, and the genomes of a larger collection of mutants were sequenced in a combined pool. This approach identified mutations in genes previously known to regulate the fMAPK pathway. These included presumed gain-of-function or activating mutations in the genes encoding the MAPKKK (Ste11p), the MAP kinase (Kss1p), and the transcription factor (Ste12p). Loss-of-function or inactivating mutations were identified in negative regulators, including one of the GTPase activating protein (GAP) for Cdc42p, Rga1p, and one of the GAPs for Ras2p, Ira2p. Alleles in previously identified negative regulators were also uncovered in *ALY1, AIM44, RCK2,* and genes that function in protein glycosylation (*KAR2* and *MNN4*). Many of the mutants contained multiple mutations, suggesting that negative regulators have additive effects on MAPK pathway activity. Understanding how negative regulatory inputs combine to modulate signaling can provide insight into the overall dynamics of MAPK pathway regulation.

534S **Analysis of the functional role of the microtubule-associated [*PUB1/SUP35*] prion-like assembly and regulation of its formation** Irina L Derkatch, Susan W Liebman, Irina Alexandrova, Dania Maldonado, Kassandra Gomez Pharmacology, University of Nevada, Reno

Prions are self-propagating protein conformations that can be acquired by various cellular proteins and then passed on to newly synthesized protein molecules with the same primary sequence. The underlying structure of most prions is amyloid, beta-sheet-rich fibers with protein molecules neatly stacked on one another. Prions and prion-like amyloid aggregates are associated with many devastating diseases, including Creutzfeldt-Jakob, Alzheimer's, Parkinson's, Huntington's, type 2 diabetes and amyotrophic lateral sclerosis. Noteworthy, most disease-associated proteins are intrinsically amyloidogenic. Mutations just increase the likelihood of forming amyloid and reduce the age of disease onset.

Saccharomyces cerevisiae is an excellent model organism for studies of prions: yeast harbor several prion forming proteins that can exist in a prion state without affecting growth rate or strain viability. Analysis of prion-forming domains reveals that many of them are rich in glutamines and asparagines (Q/N-rich) and can promote prion formation or prion-like aggregation without mutations. Retention of such domains by up to 5% of proteins is puzzling. The simplest explanation is that these proteins form functional aggregates. One possibility is that prion-like aggregation drives the assembly of membraneless organelles and large protein complexes. Indeed, the proportion of proteins with Q/N-rich domains is dramatically increased among components of P-bodies and stress granules and RNA-binding proteins. We aim to reveal functional prion-like complexes and understand the regulation of their assembly.

One candidate functional prion-like assembly is a microtubule-associated complex formed by Sup35 and Pub1 proteins encompassing Q/N-rich domains. In this complex Sup35 and Pub1 co-aggregate. Based on the preliminary analysis of other components of the complex and its importance for microtubule stability, it is hypothesized that the complex's functional role is to direct the synthesis of tubulin to the site of microtubule assembly. While testing this hypothesis we found that a spontaneous change in a yeast strain can simultaneously modulate the aggregation of one of the complex components, Sup35, and increase the stability of centromeric plasmids that depend on microtubule cytoskeleton for their transmission. We conclude that the change affects the assembly / functionality of the complex.

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535S **Fluorogen Activating Proteins are a powerful new imaging tool for quantitative protein trafficking studies in *S.***

cerevisiae Katherine G Oppenhiemer[1], Natalie A. Hager[1], Ceara K. McAtee[1], Chaowei Shang[1], Jeffrey L. Brodsky[1], Derek C. Prosser[2], Adam V. Kwiatkowski[3], Allyson F. ODonnell[1] [1]Biological Sciences, University of Pittsburgh, [2]Department of Biology, Virginia Commonwealth University, [3]Department of Cell Biology, University of Pittsburgh

Advances in genetically encoded fluorescent probes led to development of fluorogen-activating proteins (FAPs). This technology has two components: a non-fluorescent single chain antibody (SCA), which is fused to the protein of interest, and a fluorogen, which in this case is a malachite-green (MG) dye derivative that is non-fluorescent. Binding of the fluorogen to the SCA results in a 20,000-fold fluorescence increase, producing a signal in the far-red range (633 nm) for live cell imaging. FAPs have advantages over traditional fluorescent proteins. Specifically, the MG-dye can be derivatized to be cell impermeant, selectively detecting SCA-tagged proteins at the cell surface and facilitating quantitative endocytic dynamics studies. Although developed in yeast, FAPs are underutilized in this system. Here we: 1) optimized the SCA sequence for yeast expression, 2) created a series of FAP-tagging plasmids, 3) generated a suite of FAP-tagged sub-cellular markers, and 4) used FAPs to assess the endocytic trafficking of yeast G-protein coupled receptor Ste3. We mapped Ste3 endocytosis post ligand stimulation, identified *cis*-acting regulatory sequences in Ste3 and demonstrated that the alpha-arrestins, a conserved class of protein trafficking adaptor, are critical for Ste3 endocytic trafficking. We have developed a useful tool for the yeast cell biology community and demonstrate its powerful capabilities for quantitative protein trafficking studies.

536S **Deformation of the cell nucleus after high-speed stretching.** Maia Garcia[1], Sam Boland[2] [1]Bemidji State University, [2]University of Minnesota

Traumatic brain injury (TBI) affects thousands of people. As of 2021, 190 people die each day of TBI. TBI and blast induced trauma can both lead to lifelong health problems. Blast-induced traumatic brain injury (bTBI) specifically can change cellular phenotypes and contractability. Knowing how bTBI and TBI affects vascular cells is incredibly important because TBI often does not cause visible wounds, making observation of the injury difficult. Using a custom technique called cellular microbial stretching, we are able to measure the effects of traumatic mechanical injury in singular vascular smooth muscle cells. We stretch vascular smooth muscle cells at different rates and then we measure the deformation of the nucleus via fluorescent imaging. The preliminary results show that the nucleus deforms during stretching, and the deformation depends on the rate of stretching. Prior works suggest that nuclear deformation affects gene expression in cells, so these results provide us insight into how TBI can affect the function of individual vascular smooth muscle cells.

537V **Balance of recycling and degradative endosomal microdomains is controlled by the oligomerization of Hsc70 co-chaperone RME-8** Anne Norris[1], Ryan Feehan[2], Joanna Slusky[2], Barth D Grant[1] [1]Rutgers, [2]Kansas University

Endosomes balance the opposing activities of degradation and recycling. This process is facilitated by recruitment into physically distinct degradative or recycling microdomains on endosomes. Endosomal sorting complexes required for transport (ESCRT) mark the degradative microdomain, while the recycling domain is marked by the retromer complex and associated proteins RME-8 and SNX-1. How these physically and functionally distinct membranes remain separated is a major question in the field of endosomal trafficking.

Two proteins RME-8 and SNX-1 keep these domains separate in part via removal of degradative component HRS/HGRS-1 from the recycling microdomain. This activity is due to recruitment and activation of chaperone Hsc70 on the endosome by the RME-8 DNAJ domain. To better understand the mechanism of RME-8 function we used mutagenesis and structural modeling of RME-8. We identified the IWN3 domain RME-8, to be important for the autoinhibitory DNAJ domain binding, with IWN3 playing a critical role in HRS uncoating activity. Combining AlphaFold structural predictions with in vivo mutation analysis of RME-8, we propose a model whereby SNX-1 controls the oligomerization status of RME-8 and hence the productive exposure of the DNAJ domain. Furthermore we provide a model of a tetrameric RME-8 structure that exists on membranes and while dimeric and monomeric forms exist in the cytosol.

538V **Multidimensional study of exosome biogenesis in human A375 melanoma cells in response to Doxorubicin** Laura Fernandez[1,2], Adriana Umaña[1], Luis Alberto Gomez Grosso[2], Susana Novoa[2] [1]Universidad Nacional de Colombia, [2]Instituto Nacional de Salud

Extracellular Vesicles (EVs) are membranous structures responsible for transporting bioactive molecules, including proteins, lipids, metabolites, and nucleic acids, to local and distant environments. The chemotherapeutic agent Doxorubicin (Doxo) is known to induce damage to distant organs from the tumor site, being the heart particularly affected. Cancer cells increase the release of EVs, like exosomes, when treated with Doxorubicin, potentially mediating cardiotoxic effects. Challenges remain in comprehending factors influencing EV biogenesis; hence, our study aims to understand how exposure to Doxorubicin impacts

the generation and release of EVs in A375 human melanoma cells.

We exposed A375 cells to 10nM Doxo to assess the intracellular exosome generation within a period ranging from 4 to 24 hours post-exposure and examined the release of small vesicles (sEV) into the culture medium after 96 hours of exposure, followed by a 24 or 48-hour medium conditioning. By Immunocytochemistry/Immunofluorescence (ICC/IF) we explored the intracellular distribution of exosomal markers (CD9, CD63, CD81, TSG101, Rab7) over time. Acidic vesicles were stained using acridine orange, while the Golgi apparatus and multivesicular bodies (MVBs) were visualized within live cells using Bodipy-TR. Nanoparticle Tracking Analysis (NTA) was utilized to determine the size and concentration of EVs.

Exposure to Doxorubicin led to a substantial increase in CD81+, CD9+, CD63+, and TSG101+ vesicles within A375 cells, particularly at 8 and 24 hours. The EV populations exhibited sizes consistent with exosomes (24 hours: 132.4 ± 5.7 nm, 48 hours: 145.0 ± 7.8 nm), with more particles in the Doxo group compared to the Control group (Fold Change: 24 hours: 4.0 ± 0.2, 48 hours: 19.4 ± 0.1). In addition, cells exposed to Doxorubicin and cultured for 48 hours showed a significantly shortened cell cycle of A375 cells and an increased production of MVBs, which could lead to a higher exosome secretion.

In conclusion, our findings suggest that exposure of A375 cells to 10nM Doxorubicin intensifies the process of exosome biogenesis and secretion. The heightened transport of biomolecules by tumoral EVs could potentially contribute to both local and distant effects induced by Doxorubicin. This may expand our understanding of cancer biology and hold significant clinical implications for cancer treatment and the reduction of its side effects.

539V **Select mitochondrial toxicants induce an enhanced RNA interference response in *C. elegans*** Clare Sparling[1], Dillon King[1,2], Joel Meyer[1] [1]Duke University, [2]National Institute of Environmental Health Sciences

Mitochondrial dysfunction activates immune responses and plays a role in many immune diseases. Given that many environmental toxicants also target mitochondria, we assessed whether mitochondrial toxicants activate immune responses in *Caenorhabditis elegans.* Studying this question serves two purposes. One, it may identify environmental toxicants that modulate immune activity. Two, probing various aspects of mitochondrial dysfunction with mechanistically distinct toxicants may provide insight into the specific characteristics of dysfunction that induce immune activation in *C. elegans.* RNA interference (RNAi) is used by *C. elegans* to defend against pathogens. Thus, enhanced RNAi was used to screen for immune activation*. Lir-1* double-stranded RNA (dsRNA) causes lethality in *C. elegans* with enhanced RNAi but fails to do so in worms with basal levels of RNAi. *C. elegans* were developmentally exposed to various mitochondrial toxicants and stressors and fed *lir-1* dsRNA to screen for an enhanced RNAi (eri) response. Antimycin A (Complex III inhibitor), rotenone (Complex I inhibitor), paraquat (redox cycler), and sodium azide (Complex IV inhibitor) exposures induced an eri phenotype in *C. elegans*. Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (uncoupler), N,N'-dicyclohexylcarbodiimide (ATP synthase inhibitor), thenoyltrifluoroacetone (Complex II inhibitor), and UVC (mtDNA damage) exposures failed to induce an eri phenotype*.* Our findings suggest that certain aspects of mitochondrial dysfunction induce an enhanced RNAi response in *C. elegans* while others do not.

540V **Analysis of calcium-regulated phosphorylation changes in fly and mouse egg activation** Jonathon M Thomalla[1,2], John C Schimenti[2], Mariana F Wolfner[1] [1]Molecular Biology and Genetics, Cornell University, [2]Biomedical Sciences, Cornell University College of Veterinary Medicine

The oocyte to embryo transition ("egg activation") switches a terminally differentiated oocyte to a totipotent zygote. A rise in cytoplasmic calcium (Ca^{2+}) in the oocyte is the conserved trigger for new translational programs, completion of meiosis, and other activation events; these events all occur without new transcription. Here, we use a dual-model approach (Drosophila and mouse) to identify how this conserved transition is regulated. In Drosophila, Ca^{2+}-activated enzymes modify the phosphorylation states of stored maternal proteins to regulate meiotic and translational processes. We compared the Drosophila egg activation phosphoproteomes to those in Xenopus laevis, the only vertebrate with an egg activation phosphoproteome. We identified conserved phospho-proteins, and we have begun to use the Drosophila model to test the importance of their phospho-regulation by engineering versions of the endogenous loci that will encode non-phosphorylatable or phosphomimetic phosphoproteins. In addition, we are exploring the upstream regulators of the phospho-changes. While one cation channel (TRPM) is necessary for the most dramatic rise of calcium during Drosophila egg activation, the phospho-modulation appears to require additional channels, which we are currently identifying. To test whether mammals follow the Drosophila phospho-regulatory paradigm described above, we have collected over 40,000 wild type (WT, C57BL/6J) mouse oocytes and activated eggs to analyze by quantitative phosphoproteomic LC-MS/MS. Our preliminary proteomics detected proteins belonging to egg activation processes, such as spindle components, cell cycle regulators, and translation factors; we also detected phosphorylated peptides belonging to phospho-modulated proteins, like Importin-alpha-2, that we found among phospho-proteins in Drosophila and Xenopus activating eggs. To test whether Ca^{2+}-activated enzymes regulate

phospho-state in activating mammalian oocytes, as in flies, we generated a mutant mouse line for the Ca^{2+}-dependent protein kinase CaMKIIg. This CaMKIIg-/- mutant is female sterile, and its eggs fail to complete meiosis, consistent with a previous report. To test whether CaMKIIg regulates the phosphoproteome as mouse oocytes activate, we will compare the CaMKIIg-/- phosphoproteome to WT. Taken together, we are leveraging the strengths of the mouse and fly model systems to interrogate foundational and detailed mechanistic aspects of egg activation.

541T **WormAtlas: New Chapters, New Data, New Worms** David H Hall[1], Nathan E Schroeder[2], Laura A Herndon[1], Catherine A Wolkow[1], Zeynep F Altun[1] [1]Neuroscience, Albert Einstein College of Medicine, [2]Crop Sciences, University of Illinois

The well-characterized anatomy of *C. elegans* has propelled its use as a model organism. Initially characterized through extensive light and electron microscopy (EM) observations, these data continue to provide insight into *C. elegans* biology. The Center for *C. elegans* Anatomy is a hub for the presentation and interpretation of anatomical data through the WormAtlas and WormImage websites. WormAtlas comprises multiple resources designed to assist users in understanding the structure of *C. elegans* and, ultimately, in interpreting their own anatomical data. During 2023, WormAtlas and WormImage logged over 260,000 pageviews from 43,000 users. Users came from 172 countries, with the U.S. having the largest user base, followed by Germany, Japan, India, China and Canada. The WormAtlas handbook chapters include tissue-specific descriptions of *C. elegans* anatomy. Originally, focused on the adult hermaphrodite and male, we have expanded our handbooks on dauer anatomy and structural changes that occur during aging. During the past year, new chapters were posted on muscle and the reproductive system in aging *C. elegans*. We are also extending our coverage of anatomy beyond *C. elegans* by recruiting members of the nematode community to contribute chapters on *Pristionchus pacificus* and the human parasitic nematode *Strongyloides stercoralis.* In addition to our collection of physical EM archives of *C. elegans,* mostly now available in digital form on WormImage, we have begun retrieving archival EM data sets of other nematode species. These additional data sets include approximately 50,000 negatives and prints that are being digitized and annotated for future inclusion on WormImage. We have recently partnered with the NIH-funded BossDB image repository to host datasets used for the "Mind of the Worm" (White et al., 1986). To ensure the long-term viability of WormAtlas, the Hall and Schroeder labs are working together to develop new tools and features. Together, we co-hosted a workshop recently in Glasgow on advances in EM techniques for the worm. Over the next year much of the physical archives will be transferred to the University of Illinois where they will remain accessible to the research community.

542T **A robotic platform for fully automated developmental and ageing studies in *C. elegans*: application to the genetic mapping of lifespan and mitochondrial stress response** Elena Katsyuba[1], Arwen Gao[2,3], Lazar Stojkovic[1], Gaby El Alam[3], Fabien Tâche[1], Maroun Bou Sleiman[3], Matteo Cornaglia[1], Johan Auwerx[3], Laurent Mouchiroud[1] [1]Nagi Bioscience SA, [2]Laboratory Genetic Metabolic Diseases, Amsterdam UMC, [3]Laboratory of Integrative Systems Physiology, École Polytechnique Fédérale de Lausanne

C. elegans is a powerful model organism for biomedical studies. However, the traditional protocols, which continue to be broadly used, rely on manual handling, making them labor-intensive and time-consuming. Automation of these processes would greatly benefit long-term studies of *C. elegans*. Significant progress has been achieved over the past decade in the techniques to study worm's biology: the introduction of microfluidic approaches for different assay types and the use of machine learning-based algorithms for data processing offer an increase in experimental throughput and a better control of experimental conditions.

We propose here a novel solution for automated developmental and ageing studies in *C. elegans* aggregating these new mentioned methodologies. Our microfluidic-based robotic platform is capable to fully automate all the key aspects of *C. elegans* experimentation, including worm culture, treatment, imaging, as well as data recording and analysis. The unique characteristics of the platform allow high content phenotypic studies on multiple worm populations in parallel that go beyond a simple tracing of growth or survival curves. We present here a panel of standardized bioassays allowing automated: (1) monitoring of *C. elegans* lifespan, (2) assessment of worm fitness, (3) testing of different stress responses activation and (4) identification of developmental and reproductive phenotypes that can serve as potential predictors of ageing.

To validate the performance of the assays, we mapped the genetic determinants of lifespan in a worm genetic reference population – the recombinant intercross advanced inbred lines (RIAILs). From 85 worm lines, we assessed the life-history traits on-chip, including the development time, growth dynamics, and reproduction. RIAIL lifespans, previously generated with the traditional on-plate method, exhibited large variations, and were positively correlated with developmental time on-chip. Among the top candidates obtained from QTL mapping, novel longevity modulators were identified and validated.

543T **Fourth Chromosome Resource Project: a comprehensive resource for genetic analysis in Drosophila that includes humanized stocks** Michael Stinchfield[1], Brandon Weasner[2], Bonnie Weasner[2], David Zhitomersky[3], Justin Kumar[2], Michael O'Connor[3], Stuart Newfeld[1] [1]Arizona State Univ, [2]Biology, Indiana University, [3]Genetics, Cell, Development, University

Minnesota

The 4th chromosome is the final frontier for genetic analysis in Drosophila. Small, heterochromatic and devoid of recombination the 4th has long been ignored. Nevertheless, its long arm contains 79 protein coding genes. The Fourth Chromosome Resource Project (FCRP) has a goal of facilitating the investigation of genes on this neglected chromosome. The project has 446 stocks publicly available at the Bloomington and Kyoto stock centers with phenotypic data curated by the FlyBase and FlyPush resources. Four of the five stock sets are nearly complete: 1) UAS.fly cDNAs, 2) UAS.human homolog cDNAs, 3) gene trap mutants and protein traps, plus 4) stocks promoting meiotic and mitotic recombination on the 4th. Ongoing is mutagenesis of each 4th gene on a new FRT bearing chromosome for marked single cell clones. Beyond flies, FCRP facilitates the creation and analysis of humanized fly stocks. These provide opportunities to apply Drosophila genetics to the analysis of human gene interaction and function. In addition, the FCRP provides investigators with confidence through stock validation and an incentive via phenotyping to tackle genes on the 4th that have never been studied. Taken together, FCRP stocks will facilitate all manner of genetic and molecular studies. The resource is readily available to researchers to enhance our understanding of metazoan biology, including conserved molecular mechanisms underlying health and disease.

544T **Deciphering the contribution of 3D interactions between cis-regulatory elements and promoters to regulate gene expression using graph neural networks** Yang Chen[1], Elissa Lei[2] [1]LGB, NIH, [2]LBG, NIH

Gene expression is regulated by various factors including histone modifications (HMs), binding of transcription factors (TFs), and interplay of diverse cis-regulatory elements (CREs) in a cooperative manner. Two previous methods (GC-MERGE and Chromoformer) have been developed to predict gene expression by constructing a promoter-centered network based on 3D physical contacts between the promoter and CREs. However, these published models lack transferability to various sizes of gene graphs and explanation of the influence of these factors on gene expression.

We present a novel deep learning architecture creGNN based on a graph transformer network. Our approach can ascertain the key factors that regulate gene expression and can identify important CREs for each gene. We pre-trained the model using data augmentation and contrastive learning. We used ~800 ChIP-seq datasets of HMs and TFs, Hi-C/micro-C, ATAC-seq and RNA-seq data from human, mouse and fly genomes. Promoter-centered graphs were built for each gene based on the regions across the genome that contact the promoter in 3D space.

We trained a model based on HMs and calculated the Pearson correlation, AUC, and AUPRC between raw gene expression and predicted gene expression in four cell lines. creGNN showed better prediction performance relative to other existing methods. Second, we trained another model based on TFs, which showed close performance relative to that using HMs. By ranking node importance, we found that TFs and HMs co-regulate gene expression by binding to different CREs. Third, we tested the effect of 3D contacts on gene expression. 3D contact frequency showed low correlation with attention scores for all genes on a global level. Our results suggest that gene expression is dictated by promoter region activation or repression by CREs and that 3D contacts are important/necessary in specific cases but provide a lower relative contribution to overall gene expression than promoters and CREs themselves.

Compared to previously developed methods, creGNN exhibited higher accuracy and good transferability. We aspire to use our method to identify novel specific 3D contacts among promoters and CREs that contribute to the regulation of specific genes. This predictive power should not only contribute to our knowledge of specific gene regulatory mechanisms on the 3D level but also allow us to design targeted strategies to modify gene expression within individual cells.

545T **Nitroreductase Assay: A new method of cell ablation in Drosophila** Gary Teeters[1], Christina Cucolo[1], Sagar Kasar[1], Sarah Siegrist[2] [1]University of Virginia, [2]Biology, University of Virginia

Genetic control of cell ablation has allowed *Drosophila* researchers to uncover many mechanisms of development and regeneration. While powerful, this method is limited in temporal control. Gal80s can be utilized, however they use oscillations between 18°C and 29°C which changes the development rate of *Drosophila*. Disruptions of development rate can lead to difficulties interpreting results from development and regeneration assays. We have developed a new method for cell ablation in *Drosophila* based on previous work done in *Danio Rerio*. This method utilizes a UAS driven Nitroreductase gene isolated from E. coli which transform nitroaromatic drugs into strong DNA cross linkers leading to cell death. This construct allows us genetic and temporal control of cell death without the need for temperature shifts. We have found this ablation system to be effective at killing multiple developing tissues of *Drosophila*, with nearly no viability defects. In addition, we have found that animals only need to be exposed to drug for 12 hours to initiate cell death. These results lead us to conclude that the nitroreductase assay is a superior method for cell ablation in development and regeneration studies.

546T **A recombination system to assemble an integrated genetic circuit at a single locus in animals** Junjie Luo[1,2], Cheng

Huang[1,3], Seung Je Woo[1], Jane Li[1], Mark J. Schnitzer[1,2,3,4,5] [1]James Clark Center, Stanford University, [2]Howard Hughes Medical Institute, [3]Department of Biology, Stanford University, [4]CNC Program, Stanford University, [5]Department of Applied Physics, Stanford University

Transgenic reporters and effectors are important tools for genetic, developmental and neuroscience experiments. To achieve highly specific expression patterns, or to use a combination of multiple reporters and effectors, it is often desirable to create transgenic animals that have multiple transgenic elements. When recombining multiple transgenic elements into an animal genome by traditional approaches, researchers generally need to screen for a strain containing all the transgenic elements, the frequency of which declines exponentially with the number of transgenes. For this reason, traditional methods of recombination are poorly suited to the generation of animals with many transgenic elements.

To overcome this limitation, we developed a set of genetic tools that we call the 'Super Recombinator' system (SuRe), which facilitates the recombination of a chosen pair of transgenes at the same locus. The SuRe system adds an adaptor sequence upstream or downstream of each transgene. These adaptors then facilitate the recombination of the two transgenes in a transgenic tandem. Because this transgenic tandem does not segregate in the subsequent recombination steps, the SuRe system exponentially accelerates the recombination process and dramatically reduces the level of effort needed to screen for a strain with all the transgenic elements.

We tested two variants of the SuRe system in *Drosophila melanogaster*, SuRe-C (SuRe based on CRISPR/Cas9) and SuRe-CR (SuRe based on CRISPR/Cas9 and recombinase). Both variants showed recombination efficiencies dramatically higher than that of natural recombination, which is zero for recombination at the same locus. SuRe-CR has a recombination efficiency of ~48% and can recombine tandems up to 2 Mbps. SuRe-C has a lower recombination efficiency (~30% for females and ~7% for males) but involves fewer enzymes and should generalize more easily to other species.

We demonstrated the use SuRe by using it to successfully concatenate multiple splitGAL4 elements to drive the joint expression of several different fluorescent voltage indicators, enabling *in vivo* imaging of spikes in several different types of neurons simultaneously. Overall, the SuRe approach represents a new strategy for creating animals that have multiple transgenic elements and that are in increasing demand for state-of-the-art experiments in genetics, neuroscience and synthetic biology.

547T **GIVIAR: GRN Inference and Visualization from Independent scATAC-seq and scRNA-seq Data** Justin Currie, Tuan Pham, James Kentro, Gunjan Singh, Kate O›Connor-Giles, Erica Larschan Brown University

Gene Regulatory Networks (GRNs) control dynamic gene expression and thus play a fundamental role in the generation and maintenance of diverse cellular states. GRN inference methods can achieve higher resolution by integrating both gene expression and chromatin accessibility data at the single cell level. This has significantly increased demand for multi-omic datasets in which gene expression (scRNA-seq) and chromatin accessibility (scATAC-seq) data are obtained from the same cell. However, generating multi-omics data is both expensive and challenging. Therefore, the ability to integrate the large number of existing independent datasets to form pseudo-multiomic datasets is of great importance. Additionally, single-cell data suffers from high dropout levels, leading to insufficient results after downstream analysis. Here, we present GIVIAR - a GRN Inference and Visualization pipeline from Independent scATAC-seq and scRNA-seq data. Our pipeline compiles existing methods for scRNA-seq dropout imputation, integration of scRNA-seq and scATAC-seq data, and multi-omic GRN inference to produce candidate GRNs. We applied our pipeline to a single-cell atlas of embryonic D. melanogaster data with the goal of identifying transcription factors responsible for orchestrating the coordinated expression of genes involved in synapse formation. Our results reveal several known regulators of synaptic genes, as well as many new candidate regulators for in vivo validation.

548T **Generating gene-specific-split-GAL4 lines from coding intronic MiMIC lines via *in vivo* crosses for *Drosophila*** Siqi April Li[1], Hongzhou Gustave Li[2], Yu-Chieh David Chen[3], Claude Desplan[1] [1]Biology, New York University, [2]New York University Shanghai, [3]New York University

Recent technical advances in *Drosophila* genetics have enabled the generation of gene-specific T2A-split-GAL4 lines, which can be used for genetic manipulation of gene expression in various experimental settings. Our lab has recently shown that gene-specific T2A-split-GAL4 lines recapitulate well with the scRNAseq data compared to the enhancer-based split-GAL4 lines. However, converting the existing MiMIC lines into gene-specific T2A-split-GAL4 lines required embryo injection of corresponding donor DNA, which is often expensive when outsourcing *Drosophila* microinjection services to a company.

This project aims to generate MiMIC split-GAL4 drivers via *in vivo* crosses, which can provide a more accessible alternative to

traditional microinjection methods and contribute to the *Drosophila* research community. The *in vivo* crosses for generating the gene-specific T2A-split-GAL4 lines involve four rounds of crosses followed by two validation steps. First, we cross female flies carrying the T2A-split-GAL4 donor sequence with male flies carrying the MiMIC insertion and select offspring that have both donor and MiMIC genetic components. Next, we cross male offspring with female flies carrying hs-Cre and vas-PhiC31 and then select male offspring that carry all three genetic components (hs-Cre, vas-PhiC31, donor, and MiMIC) from the parents. By crossing them with female double balancer genes, we can induce the expression of Cre recombinase and thus excise the split-GAL4 donor into the circular DNA, which will then be integrated into the MiMIC insertion site by the Recombinase-mediated cassette exchange (RMCE) process. We select the males that are successfully swapped by the loss of yellow marker and then set up a single male fly cross with 3-4 double balancer females to establish potential crosses. A PCR genotyping verification of integration orientation is required since the PhiC31 system does not recognize orientation and the split-GAL4 fragments can be inserted in either direction. Finally, the potential stocks with correct orientation will then be crossed with flies carrying UAS-EGFP and constitutively expressing either GAL4DBD or VP16 to test for expression of GFP to ensure the validity of the potential stock. Our work will significantly reduce the cost and increase the accessibility of generating gene-specific T2A-split-GAL4 lines, which will enable fly researchers to generate cell-type-specific genetic tools at lower cost.

549T **Video Analysis System for Behavior and Activity Assessment of Fruit Flies in High Throughput Studies** Ibraheem Farooq[1], Brian Oliver[1], Ghadi Salem[2], Marcial Garmendia-Cedillos[2], Abhishek Somenhalli[2], Nikhil Khandekar[2], Luis Argueta[2], Noah Cubert[2], Maria Jaime[3], Tom Pohida[2], Jessica Holsopple[4], Shannon Smoot[4], Thomas Kaufman[4], Jason Tennessen[4] [1]National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), [2]Instrumentation Development and Engineering Application Solutions (IDEAS), NIBIB, National Institutes of Health (NIH), [3]Exact Sciences Laboratories, [4]Department of Biology, Indiana University-Bloomington

Behavioral analysis in organisms is very valuable because it provides insight beyond just genotypic examination, however, it is generally low throughput. Therefore, we have devised a compact, high throughput, automated behavioral screening system. The video-based system reports on the activity of each fly within each well of custom designed 24-well plate. The viewing arena is comprised of a near-infrared backlight stage with programmable white light LEDs located on the perimeter of the viewing arena. Atop the stage, the custom housing plate rests on the feeding plate allowing for an interface between the holes on the bottom on each housing well and the liquid fly food located in the feeding plate wells below. Fly survival rate upon testing in this arena environment with 25 uL of food is 96% after 12 hours and 83% after 24 hours. The survival rate improves after 24 hours if food is replaced after 12 hours and if wells are loaded with larger initial food quantities. The video monitoring system is designed to perform 24-hour plus behavioral assays where flies are exposed to liquid fly food that has been spiked with one of 216 chemicals to observe behavioral differences from wild type caused as a result of those spiked compounds. The system records video from 4 different cameras which are then stored and processed via a resnet-50 that automatically labels 2D fly skeleton key points and automatically assigns behavioral predictions based on training through a large annotation set. Current work on this project focuses on improving our data set for training, developing algorithms to automatically quantify activity and behavior for each individual fly, tracking fly key points in 3D space, and implementing real-time analysis.

550T **Rat Resource and Research Center** Elizabeth C Bryda[1], Hongsheng Men[2], Daniel Davis[2], Craig L Franklin[2], James M Amos-Landgraf[2], Yuksel Agca[2], Aaron C Ericsson[2] [1]Veterinary Pathobiology, University of Missouri, [2]University of Missouri

The NIH-funded Rat Resource and Research Center (RRRC) serves as a centralized repository for maintaining/distributing rat models and providing rat-related services to the biomedical community. Currently, the RRRC has close to 600 rat lines; all are archived by cryopreservation to ensure against future loss. The RRRC distributes live animals, cryopreserved sperm/embryos, and rat embryonic stem (ES) cell lines. Quality control measures for all materials include extensive genetic validation and health monitoring. The RRRC has expertise in rat reproductive biology, colony management, health monitoring, genetic assay development/optimization, isolation of germline competent ES cell lines from transgenic rats and can partner as consultants/collaborators. Fee-for-service capabilities include a wide variety of genetic analyses, strain rederivation and cryopreservation, isolation of rat tissues, microbiota analysis and characterization of genetically engineered rats. The RRRC, in conjunction with the MU Animal Modeling Core, makes genetically engineered rat models from start to finish using a variety of state-of-the-art technologies including genome editing (*e.g.*, CRISPR/Cas9) as well as traditional methods such as random transgenesis and modified embryonic stem cell microinjection into blastocysts. Our website (www.rrrc.us) allows user-friendly navigation. Current research efforts include generation and characterization of a variety of new rat models and improvements to rat *in vitro* fertilization. The University of Missouri is home to the NIH-funded MU Mutant Mouse Resource and Research Center (MMRRC) and the National Swine Resource and Research Center (NSRRC) as well as the MU Animal Modeling Core and MU Metagenomics Center. Together, these highly collaborative groups provide a variety of animal model-related services across species to facilitate biomedical research. Funding: NIH 5P40 OD01106.

551T **Updating Uninformative Mouse Gene Symbols** Monica McAndrews, Cynthia Smith Mouse Genome Informatics, The

Jackson Laboratory

Standardization of nomenclature for mouse genes, alleles and strains facilitates the communication and integration of scientific knowledge about the laboratory mouse and supports effective and robust comparative genomic analysis. The International Committee on Standardized Genetic Nomenclature for Mice strives to develop unique and informative nomenclature for mouse genes, genetic markers, alleles, chromosome aberrations, genomic features, and strains. With some exceptions, we coordinate symbols and names of genes and genetic markers with the Human Gene Nomenclature Committee (HGNC) and the Rat Genome Database (RGD) and we reach out to interested investigators to develop appropriate nomenclature within the Nomenclature Committee's guidelines (https://www.informatics.jax.org/mgihome/nomen/gene.shtml). Taking advantage of the recently released paralogy data provided on the Alliance for Genome Resources (https://www.alliancegenome.org/) gene pages, we are focusing on giving informative nomenclature to gene models which belong in large gene families such as the zinc finger proteins and keratin associated proteins. Many potential members of these gene families have uninformative gene symbols that consist of sequence identifiers or a sequential gene model (GM#) designation. This poster will outline recent efforts to update uninformative mouse gene symbols and include them within gene family nomenclature.

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552T **Online databases and data integration in the RIKEN BioResource Research Center** Hiroshi Masuya[1], Toyoyuki Takada[1], Daiki Usuda[1], Naomi Yuhara[1], Keiko Kurihara[1], Yuri Namiki[1], Shigeru Iwase[1], Kenta Suzuki[1], Yuki Yamagata[2], Takeya Kasukawa[3], Hideya Kawaji[4], Shinya Oki[5], Tatsuya Kushida[1] [1]BioResource Research Center, RIKEN, [2]Head Office for Information Systems and Cybersecurity, RIKEN, [3]Center for Integrative Medical Sciences, RIKEN, [4]Research Center for Genome & Medical Sciences, Tokyo Metropolitan Institute of Medical Science, [5]Department of Drug Discovery Medicine, Kyoto University

Online databases are important infrastructures for facilitating the widespread, effective, and efficient use of biological genetic resources as experimental materials in life science. To improve the findability, accessibility, interoperability, and reusability of bioresource data, adding "metadata" with data integration with public biological data is one of the effective ways.

The RIKEN BioResource Research Center (BRC) is a public repository to collect, preserve, and provide experimental mice, *Arabidopsis thaliana* as a laboratory plant, cell culture lines derived from humans and animals, microorganisms, and associated genetic materials. The online catalog of these bioresources is available at https://brc.riken.jp, with a search function by resource name, species, disease, phenotype, and gene for keywords. The system is driven by an integrated knowledge base of publicly available data on bioresources, genes, orthologs, diseases, and ontologies that form a knowledge graph through Semantic Web technologies. Currently, data and metadata of 6,156 mice, 612,131 plants, 11,857 cells, 168,193 DNAs, and 19,777 microbes are openly available from https://knowledge.brc.riken.jp and searchable from https://brc.riken.jp.

Genome-centric data integration is also underway in the RIKEN BRC. We released a genomic variation database of mouse strains, MoG$^+$ (https://molossinus.brc.riken.jp/mogplus/) in which SNPs for multiple strains including Japanese wild mice-derived strains are openly available. In 2022, collaboration is started with a genome-wide integrated database for cis-regulatory elements (CRE), trans-factors, and epigenomes, INtegrated TRAnscriptional REgulation Data platform **(**INTRARED: https://www.intrared.org/) for promoting the understanding of the transcriptional regulations. Through these activities, we will contribute to improving the reproducibility of genetics studies through the expansion of the usability of bio-resources.

553T **Update of MoG+: a database of mouse genomic variations connecting bioresource and biomedical research** Toyoyuki Takada[1], Daiki Usuda[1], Tatsuya Kushida[1], Toshihiko Shiroishi[1], Takeya Kasukawa[2], Hiroshi Masuya[1] [1]RIKEN BioResource Research Center, [2]RIKEN Center for Integrative Medical Sciences

Genome information of living organisms has become indispensable not only for understanding biodiversity, evolution, and phenotypes, but also for strategic planning of biomedical research. Use of genome information of laboratory mouse (*Mus musculus*) is essential for production of genome-edited animals. The classical inbred mouse strains are known to have mosaic genomes derived from three or four different *M. musculus* subspecies. Recently, we have conducted a whole-genome resequencing of a group of 10 wild-derived inbred mouse strains originated from the above-mentioned different subspecies. More than 40 million nucleotide variations revealed from the analysis are open to public via a mouse genome database of RIKEN BRC, MoG+ (https://molossinus.brc.riken.jp/mogplus/). MoG+ enables users to visualize intuitively those nucleotide variations in genes and intergenic regions of interest for multiple mouse strains. We are also updating MoG+ to ensure high-resolution comparison of mouse and human genome variations in orthologous genes to promote comparative genomics. MoG+ has also been upgraded with new features to facilitate research in the field of medical biology, such as addition of the latest genome information for other strains, linking to publicly available genomic variants of commonly used strains,

and human disease-associated genome variants, as well as collaboration with INTRARED (https://www.intrared.org/), a data platform to identify genome variations that affect the transcriptional regulations.

554T **Spatial statistical tools applied to genome landscapes of mutations help to refine the mutational signature concept and uncover new mutational mechanisms** Kathleen A Hill[1], David Chen[1], Bin Luo[2], Joseph Butler[1], Freda Qi[1], Nicholas Boehler[3], Hailie Pavanel[3], Cornelia Tolg[4], Eva Turley[4], Reg Kulperger[3], Charmain Dean[5] [1]Biology, Western University, [2]Statistics and Actuarial Sciences, Western University, [3]Western University, [4]London Health Research Institute, [5]Waterloo University

Mutational signatures are valuable tools for classification of cancer types and the elucidation of both mutagen exposures and mutational mechanisms. However, the current conceptualization of mutational signatures fails to consider the associations between different mutation types across the spatial landscape of entire chromosomes. Novel statistical tools permit characterization of the spatial relationship between single nucleotide substitutions or variants (SNVs) and heterozygous loci and copy number variants (CNVs). We adapted the J statistic, a nonparametric test for spatial independence between genomic events, to examine the spatial associations between SNVs, heterozygosity and CNVs in contexts of germline and somatic mutagenesis. Our J statistic pipeline is packaged as an easy-to-use, standalone R package. The pipeline can be applied to a variety of statistical genomics scenarios that require tests of association between genomic regions and sets of genomic coordinates, and accepts input data produced by genotyping arrays, whole exome sequencing or whole genome sequencing. The package offers a range of features that allow users to design hypothesis-driven tests for genomic spatial association using the J statistic, including customization of the maximum genomic distance between genomic points and regions and the interval step size to test for spatial association. We applied our tool in the two case studies: 1] associations between heterozygosity and CNVs for diverse mouse genetic backgrounds differing in the extent and genome spacing of heterozygosity and 2] associations between *de novo* SNVs and CNVs with tumorigenesis and metastasis in a mouse model of breast cancer with metastasis. Our tool successfully detected nonrandom, proximal associations between heterozygosity and CNVs relevant to novel mutational mechanisms associated with meiosis and clustered heterozygosity. We also characterized distal associations between *de novo* SNVs and CNVs in the mouse model of tumorigenesis with metastasis. Mutational signatures that incorporate signature spatial landscapes of mutations are predicted to increase the resolution of mutational signatures and cancer type classifications and advance our mechanistic understanding of mutagenesis.

555T **A statistical model for inferring rates of meiotic and mitotic chromosome segregation using data from preimplantation human embryos** Qingya Yang, Sara A. Carioscia, Rajiv C. McCoy Johns Hopkins University

Embryonic arrest and miscarriage are common in humans and largely trace to the gain or loss of chromosomes (aneuploidies) during meiosis or mitosis. Preimplantation genetic testing for aneuploidy (PGT-A) seeks to improve the outcomes of in vitro fertilization (IVF) by prioritizing chromosomally normal (i.e., euploid) embryos for transfer. This procedure entails a biopsy of 5-10 cells from the trophectoderm of day 5 blastocyst-stage embryos, which are then sequenced and classified as euploid, aneuploid, or mosaic (composed of a mixture of cells with different karyotypes). It is expected that a meiotic error arising during egg formation will lead to uniform aneuploidy across all cells and that such embryos will be classified as aneuploid. In contrast, depending on the extent and spatial organization of aneuploid cells within mosaic embryos, mosaicism may or may not be detected based on the trophectoderm biopsy. Thus, while the relative incidences of euploid, aneuploid, and mosaic embryo biopsies are readily reported based on PGT-A data, the underlying rates of meiotic and mitotic errors remain unknown. Knowledge of the rates of these fundamental biological mechanisms would inform understanding of chromosome dynamics in human embryos, with implications for both the reliability and interpretation of PGT-A data as well as the basic biology underlying early human development.

To this end, we developed a simulation framework to facilitate inference of the rates of meiotic and mitotic chromosome mis-segregation in early-stage human development, testing the range of parameter space that best explains observed PGT-A data. Specifically, we simulated thousands of embryos with varying meiotic and mitotic error rates and conducted the process of multi-cell biopsy, mimicking the clinical procedure. We then applied approximate Bayesian computation (ABC) to identify the combination of meiotic and mitotic error rates that best explain the incidences of euploid, aneuploid, and mosaic biopsies reported in recent literature. Our results reveal that a 30-35% meiotic error rate and a 2-7% mitotic error rate best approximate the published data. These estimates facilitate quantitative understanding of chromosome mis-segregation rates in human preimplantation embryos, enhancing knowledge of human development and informing the use of PGT-A data in the clinic.

556T **The European Variation Archive: Genetic variation archiving and accessioning for all species** Thomas Keane, Tim Cezard, Dona Shaju, April Shen, Nithin Kumar EMBL-EBI

The European Variation Archive (EVA) is a primary open repository for archiving, accessioning, and distributing genome

variation including single nucleotide variants, short insertions and deletions (indels), and larger structural variants (SVs) in any species. The EVA has archived more than 4.2 billion variants across 2,569 studies and 250 species since its launch in 2014. A key function of the EVA as a long-term data archive is to provide globally unique persistent identifiers for all discovered variant loci (RS identifiers) so that they can be referenced in publications, cross-linked between databases, and integrated with successive reference genome builds. For recently sequenced species, the EVA provides a free resource to archive and share genetic variants for publication and cross referencing.

The EVA regularly releases versions of all the RS identifiers it creates. The fifth release (August 2023) featured 2.2 billion variants from 250 species (featuring 18 new species including European seabass, Millet, and Plasmodium vivax). Millions of loci have been updated to a newer assembly to synchronise with the assemblies supported by Ensembl and UCSC genome browsers. The release data is available primarily via FTP[1] but all the underlying RS identifiers are queryable on the EVA website[2] and the EVA REST API[3].

Other services for researchers include: standard variant annotation, calculation of population statistics, and an intuitive browser to view and download queried variants in either Variant Call Format (VCF) or Comma-Separated Value (CSV) files backed up by a comprehensive REST API to query and export variant and genotypes programmatically.

The EVA also contributes to the maintenance of the Variant Call Format (VCF) specification and has implemented a validation suite to ensure correctness of all the submissions made to the archive. This suite, as well as the rest of our software is freely available on GitHub[4] and has amassed ~7,300 downloads worldwide.

[1] ftp.ebi.ac.uk/pub/databases/eva/rs_releases/release_5/

[2] https://www.ebi.ac.uk/eva

[3] https://www.ebi.ac.uk/eva/?API

[4] https://github.com/ebivariation

557T **Improving Laboratory Animal Genetic Reporting** Lydia Teboul[1], Guillaume Pavlovic[2] [1]MRC-UKRI, [2]Institut Clinique de la Souris

The biomedical research community acknowledges the challenges of reproducibility in biomedical research and has initiated efforts to address this issue. These efforts encompass nomenclature guidelines, enhancement and pre-registration of experimental designs, and open access initiatives. Crucial to this is the establishment of common documentation standards for animal experiments. The ARRIVE guidelines outline key aspects for documenting laboratory animals used in experiments, but a need remains for more comprehensive information on the genetics of research animals. We have started an initiative, involving members of key societies and consortia (IMGS, IMPC, ISTT) to assemble guidelines for the documentation of laboratory animal genetics. We also discuss how existing resources for standardisation of nomenclature are important frameworks that support research reproducibility.

558T **Differential and conditional elimination of marked homomorphic sex chromosomes in *Aedes aegypti* for disease vector population control** Melanie Hempel[1], Austin Compton[1], Atashi Sharma[1], Azadeh Aryan[1], Wanhao Chi[2], Xiaoxi Zhuang[2], Jake Tu[1] [1]Biochemistry, Virginia Tech, [2]Neurobiology, University of Chicago

Aedes aegypti mosquitos are the major vector for multiple viruses that cause diseases including dengue, yellow fever, and Zika. In contrast to other insect species such as *Drosophila melanogaster* and anopheline mosquitos, *Ae. aegypti* have *homomorphic sex-determining chromosomes*. On chromosome 1, there is a small (1.3 Mb), dominant, male determining locus (M-locus), while the rest of the chromosome is autosomal and susceptible to recombination. Within this M-locus resides two genes essential for male development: *Nix*, which is the male determining factor that is required and sufficient for male sex development; and *myosex*, which is responsible for male flight.

We take advantage of the homomorphic nature of the sex chromosomes and the extremely low recombination surrounding the sex locus to develop new ways to achieve effective separation of the non-biting males from the females, which is a *critical* bottleneck affecting all genetic control programs. These new methods were enabled by developing incompatible sex chromosomes and/or by conditional elimination of M- or m- bearing sex chromosomes. These methods are highly efficient and provide a diverse array of possibilities including the production of non-transgenic male mosquitoes.

One of the new approaches is a system of **D**ifferential **E**limination of **Mark**ed sex chromosomes (DeMark), which uses recessive lethal alleles to eliminate all transgenic male mosquitos, producing exclusively non transgenic males for release. We have

also created multiple lines of conditional lethality – both through selection and counterselection measures – that have the potential for the conditional selection of males. These diverse technologies can be applied to a wide array of genetic biocontrol measures to maximize disease prevention while improving efficiency, cost effectiveness, and catering to the needs of various communities.

559T **Developing a molecular toolkit for ecological, evolutionary, and functional genomics in *Daphnia*** Megan Maar[1], Jake Miller[1], Michael Lynch[2], Andrew C Zelhof[1] [1]Biology, Indiana University Bloomington, [2]Biodesign Center for Mechanisms of Evolution, Arizona State University

For most model species, little is known about the natural environment, significantly limiting our understanding of the evolutionary determinants of functional biological traits. In contrast, the aquatic microcrustacean *Daphnia* have been subject to more studies in ecology and ecotoxicology than any other organism and have led to many discoveries in functional biology, however, they are largely absent from the landscape of functional research at the gene level. We seek to develop *Daphnia pulex* as a model for testing hypotheses on functional biology through experimental manipulation at the gene level, bridging the genotype-phenotype gap. Our aim is to populate the Daphnia genome with attP sites for both PhiC31-mediated attP/attB exchange and recombinase-mediated cassette exchange, as well as develop a GAL4/UAS system for *Daphnia*. We are also generating and testing the delivery of Cas9 and sgRNAs on DNA plasmids under the control of endogenous heat shock and U6 promoters to establish a set of reagents for both generation of mutants and homologous recombination. Here, we will present our data on generating transgenic animals and improving the efficiency of CRISPR/Cas9 editing of the *Daphnia* genome.

560T **Applying machine learning to elucidate environmental components of microbial extremophilic genomic signatures** Joseph W Butler[1], Pablo Millan Arias[2], Gurjit S Randhawa[3], Maximillian PM Soltysiak[1], Lila Kari[2], Kathleen A Hill[1] [1]Biology, University of Western Ontario, [2]Computer Science, University of Waterloo, [3]Mathematical and Computational Sciences, University of Prince Edward Island

Next-generation sequencing has enabled whole genome sequencing, empowering big data analysis using bioinformatics and computation tools including machine learning (ML) algorithms. ML has been demonstrated to effectively analyze DNA sequence data for many applications including comparing and classifying sequences, and analyzing compositional patterns in DNA sequences in the context of genomic signatures. The genomic signature concept describes the patterns of oligonucleotide (*k*-mer) composition pervasive across the genome and associated with taxonomic classification even to the distinctions of species and subspecies taxa. The determinants of a genomic signature are not completely understood.

Extremophiles, organisms spanning all domains of life, are adapted to survive and sometimes thrive in extreme environments. These environments impose highly selective pressures that typically restrict life, such as extreme pH or temperature. Extremophiles are industrially relevant given their robust biocatalyst potential and useful as an emergent model organism for the study of astrobiology and early earth systems. Convergent adaptations have been observed within extremophiles groups, underlain by genomic and proteomic adaptations, such as anti-freeze proteins present in psychrophiles across all domains despite their distant phylogeny. Due to the selective pressures imposed on extremophiles by extreme environments, ML algorithms were employed to shed light on an environmental impact on prokaryotic genomic signature composition, unrelated to taxonomic contributions.

ML algorithm testing was initiated with the curation of a novel dataset of prokaryotic pH- and temperature-adapted extremophiles, including 693 genome assemblies (253 archaea, 440 bacteria) as the first iteration with dataset expansion as the curation continues. Representative sequences were constructed from the genome assemblies and labeled based on their adapted environment (i.e. thermophile, alkaliphile) and taxonomic rankings (domain-species name). Six classification algorithms were employed to assess *k*-mer profiles correlated with each group, enabling their classification taxonomically and environmentally with high and medium to medium-high accuracies, respectively. *K*-mer profiles were successfully compared to previously documented proteomic and genomic adaptations. Unsupervised approaches were undertaken and corroborated the existence of an environmental component of genomic signatures.

Overall, alignment-free ML methodologies have effectively identified macro-environmental imprints on prokaryotic extremophile genomic signatures, potentially aiding in understanding the resiliencies of extremophiles and characterizing environmental traits from metagenomic studies.

561T **Reliable estimation of tree branch lengths using deep neural networks** Anton Suvorov[1], Daniel R. Schrider[2] [1]Virginia Tech, [2]University of North Carolina at Chapel Hill

A phylogenetic tree represents hypothesized evolutionary history for a set of taxa. Besides the branching patterns (i.e., tree topology), phylogenies contain information about the evolutionary distances (i.e. branch lengths) between all taxa in

the tree, which include extant taxa (external nodes) and their last common ancestors (internal nodes). In phylogenetic tree inference, the branch lengths are typically co-estimated along with other phylogenetic parameters during tree topology space exploration. There are well-known regions of the branch length parameter space where accurate estimation of phylogenetic trees is especially difficult. Several novel studies have recently demonstrated that machine learning approaches have the potential to help solve phylogenetic problems with greater accuracy and computational efficiency. In this study, as a proof of concept, we sought to explore the possibility of machine learning models to predict branch lengths. To that end, we designed several deep learning frameworks to estimate branch lengths on fixed tree topologies from multiple sequence alignments or its representations. Our results show that deep learning methods can exhibit superior performance in some difficult regions of branch length parameter space. For example, in contrast to maximum likelihood inference, which is typically used for estimating branch lengths, deep learning methods are more efficient and accurate when inferring long branches that are associated with distantly related taxa and perform well in the aforementioned challenging regions of the parameter space. Together, our findings represent a next step toward accurate, fast, and reliable phylogenetic inference with machine learning approaches.

562T **An Improved Methodology for Sperm Cryopreservation in *Xenopus*** Carl Anderson[1], Lucia Arregui[2], Nikko-Ideen Shaidani[1], Terrence Tiersch[2], Marko Horb[1] [1]Marine Biological Laboratory, [2]Aquatic Germplasm and Genetic Resources Center

Cryopreservation of sperm from X. tropicalis and X. laevis improves the utility and availability of transgenic, mutant, and wild-type lines. Preserving distinct genetically modified lines through cryopreservation expands the ability of the National Xenopus Resource (NXR) to generate new lines by reducing demand for space. Using new methods developed in collaboration with the Aquatic Germplasm and Genetic Resources Center (AGGRC), we analyze Xenopus sperm to assess concentration, viability, and motility, along with testing fertilization rate. Prior to our shift in practices focusing on early process quality control and correction, we saw greater variation in sample quality, and a bottleneck resulting from testing fertilization rate. Changes to equipment, including the addition of a controlled-rate freezer, use of French straws, and a simplified cryoprotectant media, allow us to closely control crucial stages of the cryopreservation process. By adjusting samples prior to freezing based on these assessment tools we can ensure consistency and efficacy for users. Differences we have identified in sperm concentration between individuals further reinforces the importance of assessment metrics. Through use of these tools, we increase throughput by eliminating time spent producing and testing samples with subpar concentration, low viability, or poor motility. With the incorporation of these new practices, the Xenopus community will have increased access to cryopreserved lines, greater success using cryopreserved samples, and improved ability to generate new lines at the NXR. Steps we have taken to improve our cryopreservation program are a necessary part of addressing the ever-expanding demand of Xenopus as a research model.

563T **Intra-FCY1: a novel system to identify mutations that cause protein misfolding** Natalie Quan, Yuichi Eguchi, Kerry Geiler-Samerotte Arizona State University

Protein misfolding is a common intracellular occurrence. Most mutations to coding sequences increase the propensity of the encoded protein to misfold. These misfolded molecules can have devastating effects on cells. Despite the importance of protein misfolding in human disease and protein evolution, there are fundamental questions that remain unanswered, such as, which mutations cause the most misfolding? These questions are difficult to answer partially because we lack high-throughput methods to compare the destabilizing effects of different mutations. Commonly used systems to assess the stability of mutant proteins in vivo often rely upon essential proteins as sensors, but misfolded proteins can disrupt the function of the essential protein enough to kill the cell. This makes it difficult to identify and compare mutations that cause protein misfolding using these systems. Here, we present a novel in vivo system named Intra-FCY1 that we use to identify mutations that cause misfolding of a model protein (yellow fluorescent protein (YFP)) in Saccharomyces cerevisiae. The Intra-FCY1 system utilizes two complementary fragments of the yeast cytosine deaminase Fcy1, a toxic protein, into which YFP is inserted. When YFP folds, the Fcy1 fragments associate together to reconstitute their function, conferring toxicity in media containing 5-fluorocytosine and hindering growth. But mutations that make YFP misfold abrogate Fcy1 toxicity, thus strains possessing misfolded YFP variants rise to high frequency in growth competition experiments. This makes such strains easier to study. The Intra-FCY1 system cancels localization of the protein of interest, thus can be applied to study the relative stability of mutant versions of diverse cellular proteins. Here, we confirm this method can identify novel mutations that cause misfolding, highlighting the potential for Intra-FCY1 to illuminate the relationship between protein sequence and stability.

564T **Data Integration Through Allele Curation at SGD** Edith D Wong[1], Suzi Aleksander[1], Jodi Lew-Smith[1], Robert S Nash[1], Rahi Navelkar[2], Marek S Skrzypek[1], Stacia R Engel[1], J Michael Cherry[1], The SGD Project[1] [1]Genetics Department, Stanford University, [2]Harvard University

The *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is a model organism knowledgebase that aims to provide

a complete picture of eukaryotic cellular processes by curating a comprehensive and diverse collection of data types for the budding yeast *Saccharomyces cerevisiae*. In addition to other data, we also have been capturing single mutant phenotypes, as well as importing genetic and physical protein-protein interactions from BioGRID (thebiogrid.org) to help shed light on proteins' roles in cellular processes. While we have been collecting mutant types since we started annotating mutant phenotypes, we more recently decided to capture mutant alleles broadly and systematically. Users can now search for and find alleles associated with their genes of interest. In addition, pages dedicated to single alleles are now available and include information on allele type, molecular details of the mutation, alias names, and associated references, along with all phenotype and interaction annotations where specific alleles were used. Alleles that share phenotype and/or genetic interaction annotations are graphically represented in the 'Shared Alleles' section. Users will be able explore more fully previously unlinked data types using our new allele curation model to gain additional insight into cellular processes and pathways. This work is supported by a grant from the NHGRI (U41 HG001315).

565T **Determining the functional domains of the anti-CRISPR protein AcrIIA4 using a high-throughput deletion scan** Annette Iturralde[1], Cory Weller[2], Meru Sadhu[1] [1]National Human Genome Research Institute, National Institutes of Health, [2]Center for Alzheimer's and Related Dementias, National Institutes of Health

Determining the functionality of all parts of a protein is an important step to be able to fully comprehend how each building block of a gene contributes to its general function. By implementing a series of small to large scale deletions throughout a single gene, we can determine the significance of all parts of a protein. Many observed in-frame deletions within a protein have unknown effects, while mutational scans typically focus on indel mutations exclusively. To counter this, we have developed a method in which we can generate all possible deletions within a protein in a high throughput manner and evaluate the phenotypic result to determine critical regions. We have applied this method to AcrIIA4, an anti-CRISPR protein which inhibits Cas9. As a result, we have begun defining the functionally important parts of AcrIIA4 for the inhibition of Cas9. Notably, we have observed that the majority of deletions result in a complete loss of anti-CRISPR function. However, our data suggests that there are four potentially dispensable domains of AcrIIA4, of which we are in the process of verifying.

566T **Single cell ribosome profiling of yeast on whole populations using localized tag RNA** Ishan Maduka Gammadde Hewa[1], David Granas[2], Michael A White[3], Gary D Stormo[4] [1]Washington University in St Louis, [2]Genetics, Washington University in St Louis, [3]Genetics, Washington university in St Louis, [4]Genetics, Washington University in St. Louis

The ability to assay genetic perturbations at scale is a major source of recent progress in functional genomics. The use of sequence barcodes to perform assays on pooled libraries rather than arrays of individual samples has greatly increased the scale of genetic screens and functional assays. An important cellular phenotype that is assayed in pooled genetic screens is gene expression. However, a critical bottleneck is that gene expression is measured in pooled screens using single-cell RNA-seq technologies that require physically isolating single cells. These technologies are subject to significant technical noise and have high reagent costs. A method to perform RNA-seq on pooled samples without isolating single cells would eliminate this bottleneck in pooled genetic screens.

We are developing a method for pooled transcriptome analysis using RNA barcoding by proximity ligation. This method, called SCALoP (Single Cell Analysis of Localized RNA on whole Populations) avoids the need to isolate single cells by using a barcoding "tagRNA" molecule that stably associates with a localized pool of cellular RNAs *in vivo* via an RNA aptamer. After lysis of a pooled sample, cellular RNAs are labeled by proximity ligation to the barcoded tagRNA. The sequence barcode tags the mRNA molecules of individual cells, within a population, enables the identification of the originating cell of each transcript. We demonstrated this technology in *Saccharomyces cerevisiae* with a tagRNA directed to the ribosome.

We used SCALoP to perform ribosome profiling under multiple stress conditions. Stress conditions such as higher temperature and amino acid starvation represented nearly 1000-1500 differentially expressed genes compared to its normal conditions, which we reproducibility measured with SCALoP. Experiments will be carried out to generate single cell RNA sequencing data via unique barcode carrying tag RNA per cell. Therefore, our method addresses a promising solution which enables any large-scale genetic perturbations and RNA-seq experiments to perform without requiring physical isolation of single cells.

567T **Homology and Disease curation at *Saccharomyces* Genome Database: Budding yeast as a model for eukaryotic biology** Stacia R Engel, Robert S Nash, Edith D Wong, Suzi Aleksander, Shuai Weng, Kalpana Karra, Stuart Miyasato, J. Michael Cherry Genetics, Stanford University

The foundation for much of our understanding of basic cellular biology has been learned from the budding yeast *Saccharomyces cerevisiae*, and studies with yeast have provided powerful insights into human genetic diseases and the cellular pathways in which they are involved. This utility of yeast as a model for human disease arises from the biochemical unity that underlies all forms of life. Work with humanized yeast (in which yeast genes have been replaced with human

orthologs) and humanized yeast proteins (in which key residues have been altered to match the human sequence) has demonstrated extensive conservation of ancestral functions through time and across taxa. In order to promote and support the ways in which yeast and yeast research can inform genetic medicine, the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) is providing comprehensive curation for human disease-related genes and their yeast homologs, including high quality manually curated information regarding functional complementation and conserved function. Curated information for yeast genes is displayed on Homology pages and Disease pages at SGD in ways that allow data mining and encourage innovation for researchers studying both yeast and other organisms. These efforts are part of our continuing mission to educate students, enable bench researchers, and facilitate scientific discovery. SGD is supported by a grant from the NHGRI (U41 HG001315).

568T **Optogenetic activation of hypothalamic AgRP neurons in transgenic zebrafish larvae increased food intake** Pushkar Bansal, Erica E. Jung Mechanical Engineering, University of Illinois at Chicago

Agouti Related Protein (AgRP) neurons are located in the hypothalamus and upon stimulation, these neurons regulate hunger and hunger-mediated behaviors especially food-seeking and compulsive eating. AgRP neurons are naturally activated by ghrelin-binding onto the ghrelin receptors on the neuron surface during starvation or fasting state to evoke the aforementioned behaviors. In this study, we used channelrhodopsin (chR2), an optogenetic actuator to control AgRP neuron activity. For the first time, we observed feeding behavior in zebrafish larvae by optogenetically triggering AgRP neurons. We created a transgenic line, *Tg(agrp1:ChR2-Kaede*), where ChR2-Kaede is expressed in AgRP1 neurons. Transgenic zebrafish *Tg(agrp1:ChR2-Kaede)* larvae at 6 days post fertilization and wild-type (ABWT) larvae were used to compare the suction behavior. The food-intake response was recorded as food suction behavior in the partially immobilized larvae with mouth left to move freely and covered the larvae with fish water containing suspended food-particles. The suction-mediated food-particle movement (particle speed) was analyzed and quantified using Particle Image Velocimetry (PIV), which visualizes the traces of individual food particles. Our results show that acute post-photoactivation of AgRP-chR2-Kaede neuron complex increased food-suction behavior in transgenic zebrafish larvae compared to wild type larvae. These findings in this novel transgenic zebrafish model would be useful in studying various hunger-related behaviors, their underlying neural circuits and substrates subjected to different chemical stimuli including drugs of abuse

569T **A fluorescence integrated Zebrafish Larvae screening platform for whole body imaging** Yongwoon Kim, Madelyn Davis, Deborah Frenkel, Tom Mullins, Yifei Wang, Mariya Lomakina, Chris Bogan, Rock Pulak Union Biometrica, Inc.

Traditional techniques of preparing zebrafish larvae for imaging involve mounting them in agarose and manually rotating them to the desired viewing angle for microscopy. This process can be time consuming and tedious. Consistency is often dependent on the scientists' dexterity and focus. The throughput rate then limits the type and scale of possible experiments. The VAST BioImager is a high throughput screening platform capable of automatically handling and rotating larvae to desired positions and angles at a rate of 1 larva every 1.0 - 1.5 min at its fastest. A limitation of this system is the lack of fluorescence imaging. Typically, this is dealt with by combining the VAST BioImager with a fluorescent microscope. However, there are difficulties latent in integrating these two systems together such as microscope platform and software compatibility, and hardware constraints which can restrict activating or changing fluorescence filters to be a manual process rather than an automatic one.

Improving on the previous design of the VAST BioImager, zebrafish larvae can now be imaged with automated 3 color fluorescence plus brightfield and a 2.5x lens, enabling an all-in-one whole body imaging platform. This type of platform can be used to screen large numbers of zebrafish larvae as well as to evaluate any fluorescence properties across the entire length of the body. Rapid, repeatable rotations enable high throughput generation of optical projection tomography datasets (3D volumes of regions of interest). This whole-body imaging platform can then be used in a number of ways such as assaying development in an environmental contaminant screen or tracking cancer xenograft recession after exposure to different drugs.

570T **The International Mouse Phenotyping Consortium: a catalogue of mammalian gene function** Kalliopi Kostelidou[1], Stacy Brody[2], IMPC Consortium[1], Maya VanZanten[3] [1]IMPC, [2]National Institutes of Health Library, [3]National Human Genome Research Institute, National Institutes of Health

The International Mouse Phenotyping Consortium (IMPC mousephenotype.org) is an international effort by 21 research institutions to identify the function of every protein-coding gene in the mouse genome. The IMPC's mission is to create a comprehensive, standardised catalogue of mammalian gene function that is freely available for researchers. To achieve this, the IMPC is knocking out each of the roughly 20,000 genes that make up the mouse genome.

IMPC data is used to investigate basic biology mechanisms that can lead to new therapeutic targets or to narrow down a suspected list of genes in patients in rare or complex disease studies. IMPC has made major gene discoveries in deafness, diabetes, circadian rhythms, metabolism, vision, pain and rare disease genetics.

IMPC data is standardised, mutant phenotypes are annotated with mammalian phenotype (MP) ontology terms, and is highly quality controlled by experts and analysed using a reproducible analysis pipeline using the OpenStats BioConductor package. Data is released biannually via the IMPC portal (mousephenotype.org) and is also made available from the Mouse Genome Database and OpenTargets for model organism and translational users respectively. Data Release 20.1 (December 2023) comprises 9,393 phenotyped lines corresponding to 8,707 human orthologs.

IMPC's impact is tracked through curation of the scientific literature and via bibliometric analysis from the National Library of Medicine. These reveal >250,000 citations and ~7000 publications which use IMPC data or IMPC materials (ES cells, lines etc). IMPC will complete an additional 1800 gene knockouts by 2027.

571T **A synthetic biology approach for optimizing insulin production in yeast** Anna Rico, Lisa Scheifele Biology, Loyola University Maryland

Since the initial genetic engineering and recombinant expression of human insulin, pharmaceutical companies have developed more effective insulin analogs with fewer side effects. However, the patenting of these proteins and the processes by which to express them has allowed the insulin market to become an oligopoly and the price of insulin to rise to levels that are unaffordable to many who rely on insulin as life-sustaining medicine. The Open Insulin Foundation seeks to develop open-source genetic constructs and production methods that would enable the production and sale of the most advanced insulin analogs at prices just above the manufacturing cost. In conjunction with the Foundation, we have worked to optimize the production of a long-acting analog, glargine, in *Komagataella pastoris (Pichia pastoris)* cells. Using a synthetic biology approach, we have systematically tested and optimized promoters and secretion signals. Combined with improvements in media formulation, these have collectively raised the yield of glargine substantially over the most commonly used expression systems (the pAOX1 promoter and αMF secretion signal). While certain promoters and secretion signals were generally strong or weak, we revealed that the context in which they are expressed could significantly modulate their strengths and that the optimal combination of promoter and secretion signal needs to be experimentally determined for each new coding sequence tested. Taken together, these results demonstrate the utility of standardized and high-throughput approaches for the optimization of protein expression and the small-scale production of medically relevant biologics.

572T **Shining Light on Calcium-Mediated Morphogenesis: Forward Engineering Organ Development with Optogenetics and Mechanosensation** Mayesha S Mim[1], Nilay Kumar[1], Jeremiah Zartman[2] [1]University of Notre Dame, [2]Chemical and Biomolecular Engineering, University of Notre Dame

Cells communicate with each other to coordinate cellular processes across tissues during cell differentiation and tissue morphogenesis. This coded communication, facilitated by Calcium (Ca^{2+}) as a second messenger, controls cell mechanics to shape the growth of organs. Many unresolved questions remain about the complex morphogenetic process due to challenges in precisely perturbing and measuring cell mechanics and signaling dynamics. Thus, the overarching goal of this work is to program Ca^{2+} to elucidate the multi-layered steps of morphogenesis. To bridge this knowledge gap, we employed 1) CsChrimson as an optogenetic and 2) Piezo as a mechanosensitive tool to define relationships between signal transduction and downstream cellular responses during *Drosophila* larval development. These tools control intracellular Ca^{2+} levels at the single-cell or whole-tissue scale to direct subsequent cellular processes and define morphogenesis. We demonstrate that these channels regulate cytosolic Ca^{2+} dynamics, impacting the activities and growth-related biological pathways of several downstream proteins. We used Ca^{2+} indicator fluorescent proteins like GCaMP6f to study live Ca^{2+} dynamics, while fluorescent tagging through fixed tissue imaging provided information about subcellular processes and cytoskeletal regulation during tissue growth. Cumulatively, these findings will provide insights into the general design principles that spatiotemporally govern morphogenesis and practical applications for modulating the development of tissues and organs.

573F **TrakBox: An automated nematode tracking and analysis system** Christopher James[1,2] [1]EMbody Biosignals Ltd., [2]Biomedical Engineering Institute, University of Warwick

TrakBox is a low cost tracking system to track nematodes under a microscope, over long periods of time, and to automatically analyse and report on their behaviour. The system is made up of two parts; the low cost hardware which comprises an off the shelf USB microscope mounted on a tracking platform, and bespoke software that allows the tracking of single nematodes over long periods of time, and performs automatic analysis of behaviour. TrakBox will accommodate dishes of various sizes (as well as multi-well plates), provides its own white (LED) light source and it powered from a single USB connection (LED light source requires its own USB power source for maximum brightness).

Using off-the-shelf parts and bespoke hardware components keeps development costs (and hence final product costs) low. The tracking and analysis code is machine learning based and automatically produces high-fidelity objective data from consecutively recorded images. Whilst TrakBox can track individual worms and produce a multitude of behavioural

characteristics after analysis, it can also perform dispersal analysis as well as analysis on multiple experiments in multiwell plates.

574F **A high-throughput method for testing the impact of chemical exposure on *Drosophila* fecundity** Andreana Gomez[1], Sergio Gonzalez[2], Jaiyu Luo[2], Ashwini Oke[1], Raymond Esquerra[2], Jennifer Fung[1], Thomas Zimmerman[3], Sara Capponi[3], Todd Nystul[1] [1]UC San Francisco, [2]San Francisco State University, [3]IBM

Measurements of Drosophila fecundity are used in a wide variety of studies, such as investigations of aging, stem cell biology, nutrition, behavior, and toxicology. Fecundity assays have broad applicability because they are a readout for so many different aspects of adult fly biology, including inputs from the reproductive tract, intestine, and brain. In addition, because fecundity assays are performed on live flies, they are suitable for longitudinal studies such as investigations of aging or prolonged chemical exposure. However, because the rate of egg laying is so closely tied to the physiological state and environmental cues, fecundity assays must be carefully designed to ensure robust and reproducible results, and there are distinct challenges that arise when scaling this assay up to test multiple experimental conditions at once. We have overcome these challenges by combining a new multiwell fly culture strategy with a novel 3D-printed fly transfer device to rapidly and accurately transfer flies from one plate to another; a low-cost, custom built robotic camera to automatically capture images of the wells; and an image segmentation pipeline to automatically identify and quantify eggs. We show that this method is compatible with robust and consistent egg laying throughout the assay period, that the automated pipeline for quantifying fecundity is very accurate (r^2 = 0.98 for the correlation between the automated egg counts and the ground truth); and that this method can be used to detect effects on fecundity induce by dietary exposure to chemicals. Taken together, this strategy substantially increases the efficiency and reproducibility of high throughput egg laying assays that require exposing flies to multiple different media conditions. Our findings demonstrate a proof of principle for this approach, and we are currently using it to test for chemicals that are commonly found in modern households as well as in tobacco smoke that may pose a risk to reproductive health.

575F **Nickases and anti-CRISPR boost mosaic analysis in *Drosophila* by MAGIC** Yifan Shen[1], Ann Yeung[2], Rhiannon Clements[2], Michael Sheen[2], Kathy Wang[2], Chun Han[2] [1]Chemistry and Chemical Biology, Cornell University, [2]Molecular Biology and Genetics, Cornell University

Mosaic analysis is a powerful approach for studying tissue-specific gene function and cell-cell interaction in vivo. Besides Flippase (Flp)/FRT-based mosaic methods, we have previously developed a novel technique called Mosaic Analysis by gRNA-Induced Crossing-over (MAGIC), which utilizes CRISPR/Cas9 to induce homology-directed repair (HDR) and chromosomal crossover at a predetermined target site(s), thus avoiding the need for a FRT site. While developing and using MAGIC, we noticed some limitations: First, double strand breaks (DSBs) generated by Cas9 create a high frequency of non-homologous end-joining (NHEJ) events that mutate the target sequence, preventing subsequent cutting from occurring, and cause chromosomal aberrations, resulting in abnormal clones and cell ablation. Second, tissue specificity of Cas9 needs to be improved for more consistent clone induction. To avoid problems associated with DSBs, we explore the use of Cas9-derived mutant nickases, which create single strand cuts (nicks) and thus do not alter the original sequence. Using our existing gRNA-marker transgenes, we found that nickases can induce MAGIC clones. Currently, we are determining the factors influencing the efficiency of clone induction by nickases and comparing the frequency of cell ablation caused by nickases and Cas9. At the same time, we are applying tissue specific anti-CRISPR to increase Cas9 specificity. These attempts and results in this study will provide important insights for us to further optimize the MAGIC technique and benefit the Drosophila community by providing versatile and powerful tools for genome-wide and tissue-specific mosaic analysis.

576F **New technologies and resources from the Drosophila Research & Screening Center-Biomedical Technology Research Resource (DRSC-BTRR)** Stephanie Mohr[1], Muhammad Ahmad[1], Justin A Bosch[1], Matthew Butnaru[1], Adam Carte[1], Weihang Chen[1], Aram Comjean[1], Ben Ewen-Campen[1], Elizabeth Filine[1], Corey Foreman[1], Srishti Goswami[1], Myeonghoon Han[1], Yousuf Hashmi[1], Yanhui Hu[1], Neha Joshi[1], Ah-Ram Kim[1], Lu-Ping Liu[1], Raphael Lopes[1], Enzo Mameli[1], William Mckenna[1], Karim Rahimi[1], Alexandria Risbeck[1], Emily Stoneburner[1], Raghuvir Viswanatha[1], Baolong Xia[1], Jonathan Zirin[1], Norbert Perrimon[1,2] [1]Harvard Medical School, [2]Howard Hughes Medical Institute

The Drosophila Research & Screening Center-Biomedical Technology Research Resource (DRSC-BTRR) at Harvard Medical School (NIH NIGMS GM132087), the current iteration of what was originally founded as the Drosophila RNAi Screening Center and Transgenic RNAi Project (DRSC/TRiP), focuses on the development, optimization, and dissemination of technologies for the benefit of the *Drosophila* and broader research communities. Our areas of emphasis include (i) high-throughput screening in *Drosophila* and other insect cultured cells using CRISPR and other technologies; (ii) molecular genetic and protein technologies for use *in vivo* in *Drosophila*; and (iii) online resources for reagent design, ortholog identification, and other applications in *Drosophila*, common model species, and a growing number of arthropod species. Here, we will emphasize newly developed technologies and resources that facilitate use of cell culture to rapidly and efficiently interrogate topics that

can then be followed-up in vivo. We will also present how using *Drosophila* cells as a platform for technology testing and optimization has opened new doors to small- and large-scale studies that directly interrogate gene function in mosquitos or other non-model species. Altogether, this work is empowering studies relevant to a wide array of biological and biomedical topics, including studies of host-pathogen interactions, signal transduction, and other topics.

577F **Building Cell-type-specific Split-GAL4 Genetic Reagents Targeting *doublesex+* Neurons in Drosophila Central Nervous System** Hongzhou Gustave Li[1], Siqi April Li[2], Yu-Chieh David Chen[2], Claude Desplan[2] [1]New York University Shanghai, [2]New York University

A central goal in neuroscience is to understand how various neurons form neural circuits that produce complex innate behaviors, such as sexual dimorphic behaviors. The *doublesex* (*dsx*) gene is critical for *Drosophila* sexual dimorphic behaviors, such as female receptivity and male courtship song. Creating cell-type-specific tools for doublesex neurons facilitates our understanding of neural diversity and the role of individual *dsx*$^+$ cell types in sexually dimorphic behaviors.

The Desplan Lab recently developed the gene-specific split-GAL4 system that accurately labels specific neuron cell types in the *Drosophila* visual system in a predictable manner. One of the important features is that these gene-specific split-GAL4 lines can be used in other tissues as long as the genes are expressed in the tissue of interest. In this study, we collaborated with Dr. Yun Ding's lab (UPenn) to explore their single-cell RNA sequencing dataset of all *dsx*$^+$ neurons and designed a genetic crossing scheme to generate gene-specific split-GAL4 reagents targeting individual *dsx*$^+$ clusters/cell types.

We first selected the target gene pairs that label specific clusters based on the transcriptomic dataset of *dsx*$^+$ neurons. We leveraged the publicly available coding-intron MiMIC insertion lines and the T2A-split-GAL4 donor lines to insert the T2A-split-GAL4 donor to the target gene via trojan exon Recombinase-mediated Cassette Exchange (RMCE). The T2A-split-GAL4 construct was excised from the original locus and circularized by Cre recombinase, followed by in-vivo swapping with MiMIC via phiC31 integrase. As RMCE does not have orientation preference, genotyping PCR was performed to screen for flies with split-GAL4 insertion orientation the same as the target gene orientation. We have generated four gene-specific split-GAL4 lines and will test the validity of these split-GAL4 lines by crossing with flies carrying dsx-flp, UAS-FRT-STOP-FRT-EGFP constructs, and pan-neuronal DBD/AD hemi-driver. EGFP expression will be examined under fluorescence microscope.

Our work demonstrates the feasibility of generating gene-specific split-GAL4 lines by the Trojan-exon-mediated in vivo swapping scheme. It offers a cost-effective alternative to traditional microinjection methods for converting coding intronic MiMIC lines into gene-specific split-GAL4 lines. The split-GAL4 lines generated in this study will benefit the Drosophila *doublesex* neuron research community by providing genetic tools to label and manipulate individual *dsx* neuron types in the central brain.

578F **MLDAAPP: Machine Learning Data Acquisition for Assessing Population Phenotypes** Amir R Gabidulin Biology, Washington State University

MLDAAPP is a computer vision machine learning based script that utilizes a state of the art open-source machine learning algorithm called YOLOv8. The script is designed to assist in quantifying phenotypical traits of model organisms by reducing the time investment needed to collect data and opening new pathways to more intricate and efficient experimental designs. MLDAAPP uses cheaply available resources, and mainly focuses on collecting empirical data from non-standardized environments. It is an easy-to-implement method, that requires little to no investment in both time and monetary costs. MLDAAPP has already been successfully implemented in assessing various phenotypes such as locomotion, size, and count in *Drosophila melanogaster,* setting up the basis of its viability for current and future work.

579F **Optical system with blue LED light for multiple studies in *Drosophila melanogaster* cultures** Monica Andrea Lopez Bautista[1], Viviana Matilde Mesa Cornejo[1], Jorge Enrique Mejía Sánchez[1], Francisco Javier Ornelas Rodríguez[2] [1]Centro Universitario de los Lagos, Universidad de Guadalajara, [2]Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada, Instituto Politécnico Nacional

Drosophila melanogaster has become a model organism widely studied for more than a hundred years by various research groups that has allowed to understand many behaviors, especially the circadian cycle, which regulates sleep-wake conditions in a 24-hour cycle day and depends closely on exposure to blue light short wavelength. Blue light represents the main entrainment signal of circadian rhythms and has been directly involved in the suppression of melatonin in humans as well as the extracellular and intracellular photosensitized production of reactive oxygen species (ROS), generating various general health implications. But one of the main drawbacks when carrying out experiments using this model organism exposed to light is the limitation of the population number that can be obtained. Therefore, in this work we present an optical assembly with a hexagonal arrangement equipped with blue LED light ($\boldsymbol{\lambda}$: 457.33 ± 45 nm) for the homogeneous irradiation of fly cultures,

which can be used for various applications depending on the study to be carried out; from retinal degeneration, longevity, reproduction, courtship, among others. For this arrangement, a space of 1.6 x 1.7 x 0.2 m was used, which resulted in the assembly of 95 blue light LEDs that provided us with a capacity of up to 153 flasks or fly cultures, which can be arranged randomly, allowing the development of assays of more than 5 generations with 5 repetitions in two strains simultaneously, depending on the experimental design. The illuminance of this assembly from 0 to 601.12 Lux can be programmed with the change of current injected into the system from the variable power source from 0 to 1.05 A, respectively. Its construction is cost-effective, providing a great tool that allows easy manipulation of a large population of flies across various generations and strains. In addition, it is programmed on a 12-hour light, 12-hour dark cycle with a timer in real time, with controlled temperature and humidity.

580F **New CRISPR-induced, loss-of-function mutations from the Fourth Chromosome Resource Project enabling clonal analysis of fourth chromosome protein-coding genes** Bonnie Weasner[1], Brandon Weasner[1], Robert Eisman[1], Kevin Cook[1], Justin Kumar[1], Stuart Newfeld[2] [1]Indiana University, [2]Arizona State University

The Fourth Chromosome Resources Project is generating stock resources for the analysis of fourth chromosome genes. One of our efforts is to induce at least one mutation in every protein-coding gene on an FRT-bearing fourth chromosome using CRISPR mutagenesis with a single guide RNA and to provide preliminary phenotypic characterizations of the new mutations. Our methods assure that the new mutations are established in a single genetic background, making these high-quality stocks particularly well suited for the analysis of background-sensitive phenotypes such as behavior and the influence of chromatin conformation on gene expression. We will present our progress in completing the screens, our molecular analyses of the new mutations, our assessments of recessive lethal, sterile and morphological phenotypes, our quality-control tests and our preliminary characterizations of eye-antennal imaginal disc phenotypes in FLP-induced mitotic clones. Completion of this work will provide a comprehensive set of molecularly characterized, loss-of-function mutations for the entire fourth chromosome.

581F **Predicting gene expression dynamics in *D. melanogaster* through super resolution live imaging and computational modeling system** Priyanshi Borad[1], Parisa Boodaghimalidarreh[2], Biraaj Rout[2], Mohammad Sadegh Nasr[2], Md Jillur Rahman Saurav[3], Kelli Fenelon[4], Jacob Luber[5], Theodora Koromila[6] [1]Biology, University of Texas at Arlington, [2]Computer Science, University of Texas at Arlington, [3]University of Texas at Arlington, [4]University of Texas Arlington, [5]Computer Science, University of Texas Arlington, [6]Biology, University of Texas Arlington

One of the major challenges of the modern genomics era is to better understand how gene expression is regulated to support spatiotemporal outputs that change over the course of development. It is known that multiple, transiently acting enhancers act sequentially to support changing outputs of expression for some genes. The early *Drosophila* embryo has served as a paradigm for how enhancers control patterning and has also demonstrated that the patterning process is dynamic, whereas other genes are controlled by enhancers that act over a longer period and support changing spatial outputs over time. For example, expression of the gene short gastrulation (*sog*) is driven by at least two co-acting enhancers that support temporally dynamic. Live imaging experiments offer the capacity to analyze gene expression dynamics with increased temporal resolution and linear quantification. However, genetic and live imaging techniques have outpaced analysis techniques to harvest the bountiful information contained within real-time movies of transcriptional dynamics with modern methods confined to static parameter cell and transcript tracking methods. To assess gene expression systematically, we developed a quantitative approach to measure the spatiotemporal outputs of enhancer-driven MS2-yellow reporter constructs as captured by *in vivo* imaging to provide information about the timing, levels, and spatial domains of expression. We introduce a pipeline based on Random Forest Regression to predict the future distribution of cells that are expressed by the *sog-D* gene in the *D. melanogaster*. This method provides insight about how cells and living organisms control gene expression and consists of three parts: 1) Super resolution imaging of whole embryo spatial transcriptomics imaging at sub cellular, single molecule resolution, 2) Image processing that includes resizing, segmentation, and identifying transcriptionally active cells, and 3) A Random Forest regression model that can predict the next stage active distribution based on the previous one. In conclusion, with super resolution live imaging technique and Random Forest Regression computational tool we can predict future spatially resolved gene expression using features from the spatial point processes which is analogous to RNA Velocity for spatially resolved developmental biology.

582F **Balancing competing effects of tissue growth and cytoskeletal regulation during *Drosophila* wing disc development** Nilay Kumar[1], Jennifer R Ambriz[2], Kevin Tsai[2], Mayesha S Mim[3], Marycruz Flores Flores[3], Weitao Chen[2], Mark Alber[2], Jeremiah Zartman[3] [1]Chemical and Biomolecular engineering, University of Notre Dame, [2]University of California, [3]University of Notre Dame

How a developing organ robustly coordinates the cellular mechanics and growth to reach a final size and shape remains poorly understood. Through iterations between experiments and new model simulations that include a mechanistic description of

interkinetic nuclear migration, we show that the local curvature, height, and nuclear positioning of cells in the *Drosophila* wing imaginal disc are defined by the concurrent patterning of actomyosin contractility, cell-ECM adhesion, ECM stiffness, and interfacial membrane tension. We show that increasing cell proliferation via different growth-promoting pathways results in two distinct phenotypes. Triggering proliferation through insulin signaling increases basal curvature, but an increase in growth through Dpp signaling and Myc causes tissue flattening. These distinct phenotypic outcomes arise from differences in how each growth pathway regulates the cellular cytoskeleton, including contractility and cell-ECM adhesion. The coupled regulation of proliferation and cytoskeletal regulators is a general strategy to meet the multiple context-dependent criteria defining tissue morphogenesis.

583F **Homeostatic behavior quantification of mice in a social context using machine vision** Jessica D Choi[1,2], Cayson Hamilton[3], Brian Geuther[4], Gautam Sabnis[4], Tom Sproule[4], Vivek Kumar[4] [1]Genetics, The Jackson Laboratory, [2]Tufts University, [3]Brigham Young University, [4]The Jackson Laboratory

Understanding nuances in feeding and drinking behaviors is crucial when assessing disorders such as obesity, diabetes, and metabolic syndrome. Obtaining a reliable and efficient method to assess these behaviors in mice can provide powerful insight into the effects of these disorders. Technological advances in the computer vision field have enabled studying mouse behavior with a higher degree of sensitivity. Our group has applied these techniques to study mice in a social context, which allows for more naturalistic behavior in group-housed animals with the potential to produce more relevant data. Using machine vision over 46 long-term, continuous four-day experiments with three mice in each arena, I capture feeding and drinking behaviors across the classic inbred strain C57BL/6J, the autism spectrum disorder model BTBR *T+ Itpr3^{tf}*/J (BTBR), heterozygous B6.BKS(D)-*Lepr^{db}*/J (Lepr) control mice, and homozygous Lepr diabetic mice.

We trained machine learning (ML) classifiers for feeding and drinking through supervised learning. To validate the drinking classifier, I ran a Lickometer experiment, which is a mechanical solution to measuring drinking behavior, that logs contact events with the water spout. I manually scored each contact bout as a drinking bout or a non-drinking contact bout. We validated the feeding classifier through a classical ML approach of densely annotating videos. We used these datasets as ground truth to measure precision, recall, and F1 scores.

Because BTBR mice are used as a model of autism spectrum disorder, we compared social feeding behavior where mice eat at overlapping times from the same food hopper. Interestingly, we find the C57BL/6J mice participating in more social feeding bouts with longer durations compared to BTBR mice, aligning with the BTBR strain's known antisocial tendencies, which have been previously characterized.

We also compared heterozygous and homozygous Lepr mice in terms of their feeding, drinking, and general activity. Activity levels are typically used as a measure of circadian rhythm. However, while homozygous Lepr mice have circadian rhythms that are extremely arrhythmic when looking at activity, they preserve diurnal feeding and drinking rhythms. This highlights the usefulness of measuring other homeostatic behaviors to glean circadian insights of less active mouse strains. These novel ML tools enable a new generation of assays that can use group-housed mice to produce more naturalistic data.

584F **Long-read sequencing with adaptive sampling for characterization of CRISPR/Cas9-generated transgenic mice** Zachary T Freeman[1], Weisheng Wu[2], Laura Burger[3], Elizabeth Hughes[1], Thomas L Saunders[1], Shipra Garg[4], Olivia Koues[4], Judith S Meyers[4], Suzanne M Moenter[5], Chris Gates[2] [1]Transgenic Animal Model Core, University of Michigan, [2]Bioinformatics Core, University of Michigan, [3]Molecular & Integrative Physiology, University of Michigan, [4]Advanced Genomics Core, University of Michigan, [5]Molecular and Integrative Physiology, University of Michigan

Complete characterization of CRISPR/Cas9-generated animal models presents many challenges that may require advanced sequencing methods. We previously used a long single stranded donor combined with a single gRNA strategy to generate an endogenous iCre knock in the *Npvf* gene in C57BL/6J mice. After breeding to wild type, candidate G1 founders were identified using a screen PCR, but spanning PCR across the insert were unsuccessful at fully characterizing the entire allele. We used Oxford Nanopore Technology (ONT) long-read sequencing (LRS) with adaptive sampling for Chromosome 6 on an individual G1 founder to determine if the entire insert sequence and arms of homology were correct as designed. Reads were aligned to mouse reference genome GRCm38 using the wf-alignment pipeline with the minimap2 v2.24 to align reads. Structural variants (SV) were then called using Sniffles and CuteSV and consensus SVs were called using SURVIVAL v1.0.7. Adaptive sequencing resulted in ~58X total with 30X coverage (51.7% allele frequency) of the targeted allele, which contained an SV at the expected site. All reads were then segregated into variant-supporting versus reference-supporting reads and assembled into separate consensus sequences using Flye v2.9.1. These two consensus sequences were then aligned individually to the megamer sequence with variant-supporting reads matching the megamer sequence in the correctly targeted location with no changes observed outside the arms of homology. We next used barcoding of 3 different G1 founders in equimolar concentrations to

examine the efficacy of LRS with adaptive sampling across pooled samples. Overall coverage decreased to 31X with a range of 8-13 reads per barcoded sample. Each barcoded sample contained only 2-3 reads with the expected SV, which was insufficient to resolve final sequence. These data support the use of ONT adaptive sequencing for the characterization of CRISPR/Cas9-generated animal models.

585F **Update of the Collaborative Cross Population in the UNC Systems Genetics Core Facility** Michelle E Allen[1,2], Madison A Drushal[1,2], Timothy A Bell[1], Matthew W. Blanchard[1,3], Jackie Brooks[1,3], Joe Farrington[1], Pablo Hock[1], Richard Austin Hodges[1,2], Darla R Miller[1,2], Ginger D Shaw[1], Fernando Pardo-Manuel de Villena[1,2,3,4], Martin T Ferris[1,2], Rachel Lynch[1,2,5], Jennifer Brennan[3] [1]Genetics, University of North Carolina at Chapel Hill, [2]Systems Genetics Core Facility, University of North Carolina at Chapel Hill, [3]MMRRC at UNC, University of North Carolina at Chapel Hill, [4]Lineberger CLineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, [5]3Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

The Collaborative Cross (CC) is a multi-parental genetic reference mouse population derived from eight founder inbred strains, encompassing an extraordinary level of genetic diversity. The mission of the UNC Systems Genetics Core Facility (SGCF) is to maintain and distribute CC mice. CC mice, and their associated genomic data, are accessible to researchers with minimal conditions of use. The SGCF distributes mice to researchers at cost and functions on a first-come, first-served basis. The CC population at the SGCF has recently undergone significant changes. First, the set of CC strains currently distributed from the SGCF and the Mutant Mouse Resource and Research Center (MMRRC) at UNC is now complete at 63 strains, and every effort is being made to avoid extinction of any of these strains. Second, the majority of these strains were rederived into UNC's highest health status Barrier Facility. As of today, we have 59 strains in this Barrier Facility, specifically noting that CC038/GeniUnc has become distributable from the Barrier Facility in the last 6 months. Finally, CC strains have become significantly more inbred in the more than 9 years since the latest genetic characterization, and the strains have also undergone recent population bottlenecks). In 2021, we used these bottlenecks to define a new set of Most Recent Common Ancestors (MRCAs), and we used these animals to update our genetic characterization of the strains. DNA from 6-44 MRCAs per CC strain were individually genotyped with the MiniMUGA array (revealing a significant reduction in residual heterozygosity, with 21 strains inbred at the 99% cut off level) and pooled for whole genome sequencing (WGS). We plan on releasing strain-specific variant information after sufficient data has been generated, and after quality control is completed. We have partnered with the MMRRC at UNC to cryopreserve a majority of the extant strains as a secure reserve in case of extinction or disaster. With the MMRRC, efforts are still underway to rederive the last 4 CC strains into the Barrier Facility, with the ultimate goal of having all CC strains distributed through the Barrier Facility. In summary, this updated, uniform description of the entire CC colony at UNC will be a valuable resource to the research community, further increasing the value of the CC population.

586F **The mouse Gene Expression Database (GXD): a tool for accelerating insights into the molecular mechanisms of development and disease** Constance M Smith, Jacqueline H Finger, Terry F Hayamizu, Ingeborg J McCright, Jingxia Xu, David R Shaw, Richard M Baldarelli, Joel E Richardson, Martin Ringwald The Jackson Laboratory

The Gene Expression Database (GXD; www.informatics.jax.org/expression.shtml) provides detailed information about when and where genes are expressed in different mouse strains and mutants, with a particular emphasis on mouse development. As part of the Mouse Genome Informatics (MGI) resource, GXD combines its expression data with genetic, functional, phenotypic, and disease-oriented data, thus fostering insights into the molecular mechanisms of development, health, and disease.

GXD integrates data from RNA *in situ* hybridization, immunohistochemistry, knock-in reporter, RT-PCR , Western blot, and RNA seq experiments. The *in situ* and blot data are obtained via curation of the published literature and through collaborations with large-scale data providers; there are currently ~2 million such expression results for ~16,000 genes, with ~460,000 accompanying images. The RNA-seq data is imported from the EBI's Expression Atlas. The Expression Atlas generates uniformly processed TPM-level data sets for a select set of high-quality, bulk RNA-seq experiments. As part of its incorporation into GXD; these data are further processed and annotated, resulting in seamless integration with GXD's *in situ* and blot data. The result is an extensive database that enables search capabilities unavailable elsewhere using a wide variety of biologically- and biomedically-relevant parameters. Analysis of results from these searches is facilitated by interactive summaries and matrix views that have advanced filtering and export capabilities. Additionally, GXD provides an RNA-Seq and Microarray Experiment Search utility to reliably search for mouse tissue and cell type experiments deposited in NCBI's GEO and EBI's ArrayExpress. Searches using this tool are effective because they use standardized sample and experiment metadata annotations done by our curators; there are currently ~6,500 such indexed experiments in GXD. GXD is supported by NIH/NICHD grant HD062499.

587F **Crispant founder mice display recessive visible phenotypes** Rebekah Tillotson[1,2,3], Julie Ruston[2], Li-Hsin Chang[3], Marina Gertsenstein[4], Lauri Lintott[2,4], Christine Taylor[2], Lauryl M. J. Nutter[2,4], Monica J. Justice[2,5] [1]MRC Human Genetics Unit, University of Edinburgh, [2]The Hospital for Sick Children, [3]University of Oxford, [4]The Centre for Phenogenomics, [5]Department of Molecular Genetics, University of Toronto

CRISPR/Cas9 genome editing technology has revolutionised animal model production. In the mouse field, the gold standard for generating knock-outs is to introduce ribonucleoprotein (RNP, comprising Cas9 protein and synthetic guide RNA) into zygotes resulting in indels via the non-homologous end joining (NHEJ) repair pathway. The resulting founder mice are bred to establish pedigrees that undergo quality control assays prior to phenotyping. In contrast, the zebrafish and Xenopus communities have employed CRISPR technology in genetic screening pipelines that assess phenotypes in founders (F0), referred to as "crispants". We investigated the usefulness of crispant mouse founders for genetic screening by knocking out genes associated with recessive visible phenotypes, such as coat colour and ear morphology. Excitingly, we found that almost all crispant mice displayed the expected phenotypes. To establish the validity of phenotypic assessment of crispants, we addressed concerns in the field about the impact of genetic mosaicism. While many of our founders carried multiple small and large indels, wild-type (unedited) sequence was almost always absent, consistent with the displayed phenotype. Additionally, several founders were compound heterozygotes carrying a null mutation and a short in-frame mutation, the latter of which facilitated functional assessment of the mutated protein domain. Our results set the stage for using CRISPR/Cas9 technology for phenotypic screening in mice.

588F **Sequencing for Dummies: Using Nextflow Pipelines to Increase RNAseq Analysis Accessibility** Katy Martinson, Susan Deering, Sarah Lower Biology, Bucknell University

Differential gene expression (DGE) analysis of RNA sequencing (RNAseq) data is a useful tool for investigating where and when different genes are active. This analysis requires multiple computational steps; for example, filtering, aligning, and normalizing the sequencing data, before it can be used for experimental analysis. With technical advances in sequencing, large RNAseq projects are more affordable and feasible in non-model organisms that lack previous genomic resources, opening up DGE analysis as a tool in many more organismal systems. To increase the accessibility of this tool, it is important to make DGE analysis more easily done by biologists who are unfamiliar with computational science. To assist in this effort, we developed a computational pipeline that automatically cleans and filters the sequences to prepare a transcriptome assembly of given short read RNAseq data, and preprocesses it for analysis in R. This pipeline parallelizes the cleaning/filtering, assembly and analysis processes across multiple samples by using Nextflow's native ability to efficiently work in computing cluster systems. It also provides computational efficiency statistics to minimize wasted resources. We benchmarked the tool using RNAseq data from wild-caught Lucidota atra fireflies, an unlighted species for which there is no reference genome. We found that this tool vastly decreased the amount of time it takes to run DGE analysis, particularly for the novice user.

589F **NCBI GEO's GEO2R tool increases access to public genomics data with new visualization features and incorporation of RNA-seq studies** Emily Clough, Tanya Barrett, Steve E Wilhite, Pierre Ledoux, Carlos Evangelista, Irene F Kim, Maxim Tomashevsky, Kimberly A Marshall, Katherine H Phillippy, Patti M Sherman, Hyeseung Lee, Naigong Zhang, Nadezhda Serova, Lukas Wagner, Vadim Zalunin, Andrey Kochergin, Alexandra Soboleva NCBI, NLM, NIH

The Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) is an international public repository that archives gene expression and epigenomics data sets generated by next-generation sequencing and microarray technologies. The GEO repository is built and maintained by the National Center for Biotechnology Information (NCBI), a division of the National Library of Medicine (NLM). In addition to archiving data, GEO provides the web-based analysis tool GEO2R that enables users to analyze data stored in GEO within minutes. GEO2R facilitates access to and analysis of genomic gene expression data by removing the burden of data download and bioinformatic computation. GEO2R has recently been updated to include a wide variety of data visualization plots and can now be used to analyze more than 23,000 human RNA-seq studies available in GEO. The new data visualization plots include Volcano, Mean-difference, UMAP, box plot and Venn diagram, among others. These plots enable users to evaluate sample relatedness and analysis results. Some plots such as Volcano, Mean-difference and Venn diagram have interactive "explore and download" features where information on genes and statistics are visible by "mouseover" of points on the plot and interesting subsets of data (such as differentially expressed genes) can be downloaded. GEO2R has been expanded to include analysis of human RNA-seq data stored in GEO using DESeq2 software. The read counts for the RNA-seq samples utilized by GEO2R are calculated from submitter-provided raw reads with the NCBI Sequence Read Archive (SRA) RNA-seq Counts Pipeline (described at https://www.ncbi.nlm.nih.gov/geo/info/rnaseqcounts.html). The raw and normalized read count matrices for human RNA-seq studies are available for download directly from the GEO website. With the expansion of GEO2R for human RNA-seq samples, approximately 50% of studies in GEO can be analyzed with GEO2R. All studies that can be analyzed with GEO2R can be identified by searching GEO DataSets using the filter "geo2r"[Filter].

590F **Error-Free Single Molecule Nanopore Sequencing to Observe Evolving Viral Quasi-Species** Adam Zahm, Caleb Cranney, Alexa Gormick, Justin G English Biochemistry, University of Utah

We present ConSeqUMI, a computational pipeline for unique molecular indexing based Nanopore sequencing of long DNA and RNA molecules with base calling error rates below those observed for high fidelity PCR reactions. We have developed a range

of biochemical and computational benchmarks to vet and internally control the run-to-run accuracy of this platform. We have subsequently applied and validated this system for a range of applications including mapping the landscape of chemically-induced PCR mutagenesis, the contiguous identity of multiple plasmids during long term evolution in bacteria, the single molecule integrity of packaged transgenic AAV, the distributed genomic mutagenesis of Sindbis virus, and the quasi-species identity of patient SARS-CoV-2 and HIV samples. We further demonstrate that any single identified molecule can be extracted from the sample library via PCR and clonally expanded for further interrogation, obviating the need for synthesis and enabling rapid isolation and characterization of many unique molecular variants in a single sample library. ConSeqUMI is publicly available and ready for immediate use.

591F **Predicting Antibiotic Resistance in E. coli Using Machine Learning Models** Pleuni S. Pennings, Melika Teimouri Biology, San Francisco State University

Background: Antibiotic resistance in *Escherichia coli* (E. coli) presents a significant public health challenge. Swift and accurate identification of drug-resistant E. coli strains is crucial for optimizing patient treatment, reducing drug administration time, and curbing the spread of resistance. Machine learning holds the potential to expedite antibiotic resistance identification, leading to more efficient patient care.

Aim: This study aims to predict antibiotic resistance in E. coli through genomics. Our primary goal is to identify the most effective machine-learning technique for predicting drug resistance in *E. coli*. A recent one-year dataset from a hospital in the US provides a unique, high-quality source of labeled *E. coli* genomes. This dataset enables us to meticulously evaluate and select the optimal machine-learning approach for improving drug resistance prediction in *E. coli.*

We will employ gradient-boosted tree algorithms and neural networks for our machine-learning analysis, informed by prior research demonstrating their effectiveness with genomic data. Our main objective is to assess whether modern machine learning methodologies can outperform traditional rule-based approaches like CARD and ResFinder and if so, for which drugs and/ or which drug-resistance mechanism. If we find that these advanced machine-learning techniques can provide more precise predictions of antibiotic resistance, this could enhance patient care and treatment decisions.

592F **Using JBrowse 2 for multi-species genome browsing for the Alliance of Genome Resources** Scott Cain, Alliance of Genome Resources Consortium Stein Lab, Ontario Institute for Cancer Research

The Alliance of Genome Resources (https://alliancegenome.org) is a database that serves multiple model organism research communities to develop and maintain tools and datasets for comparative genomics. As part of this effort, we are deploying a new genome browser using JBrowse 2 (Diesh et. al., Genome Biol. 2023 Apr 17, PMID: 37069644) to provide a multi-species browser, capable of viewing synteny and genomic data tracks across multiple species. The new JBrowse 2 instance replaces the previous JBrowse 1 browser, adding new functionality, expanded data options, and a new user interface. All the same tracks available previously have been maintained, including the MODs' curated gene sets, curated variants and high throughput variants, and a considerable amount of data has been added to the new instance, including data tracks for each assembly from their home model organism databases, as well as tracks identifying orthology between genes in other Alliance genomes. These orthology tracks make use of the synteny view provided by JBrowse 2, which compares the locations of genes on two distinct genomes. The ability to open these synteny views alongside supplementary data has resulted in a powerful data exploration tool for users of the Alliance of Genome Resources.

593F **Predicting Escherichia coli Drug Resistance through Different Deep Learning-Based Approaches using a Comprehensive Pan-genome Assembly** Estefanos Kebebew[1], Gian Carlo L Baldonado[2], Abdoulfatah M Abdillahi[2], Juvenal F Barajas[2], Anagha Kulkarni[2], Ilmi Yoon[2], Pleuni S. Pennings[1] [1]Biology, San Francisco State University, [2]Computer Science, San Francisco State University

Drug resistance poses a significant challenge in combating pathogenic infections, as many pathogens can develop mechanisms to evade the drugs designed to eliminate them. This issue is exemplified by Escherichia coli (E. coli), a bacterium residing in the intestines of healthy individuals, which exhibits a remarkable capacity to accumulate resistance genes. Prior machine-learning based studies have shown that using single nucleotide polymorphisms (SNPs) or gene-presence absence tables from genome data yielded impressive results. However, it is unknown whether the use of both SNPs and gene-presence absence tables could improve performance. With recent advancements in sequencing technology, a comprehensive pan-genome assembly, containing information about both SNPs and gene-presence absence, was constructed to be employed in different deep-learning based approaches. Here we present three of these novel approaches in leveraging deep learning models and machine learning techniques using the pan-genome assembly as inputs for predicting drug resistance in E.coli. (1) The first approach leverages a simple multi-layer perceptron. (2) Our second approach builds on transforming these pan-genome assemblies into visual colormaps (images) and employs convolutional neural networks. (3) Lastly, our third approach uses extracted latent

genetic features from these images using autoencoders. All approaches will implement a multi-label classification model that predicts drug resistance of E.coli isolates across multiple antibiotics. The result of this work will help inform doctors the most appropriate antibiotics to prescribe to their patients and pave the way for deep-learning based drug resistance prediction for other pathogens.

594F **Xenbase: latest support for genomics and disease models.** Malcolm Fisher[1], Christina James-Zorn[1], Virgilio Ponferrada[1], Taejoon Kwon[2], Andrew Bell[1], Erik Segerdell[1], Konrad Thorner[1], Ngoc Ly[1], Vy Ngo[1], Stanley Chu[3], Sergei Agalakov[3], Bradley I Arshinoff[3], Dong Zhuo Wang[3], Troy Pells[3], Vaneet Lotay[3], Kamran Karimi[3], Peter Vize[3], Aaron Zorn[1] [1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, [2]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology (UNIST), [3]Department of Biological Sciences, University of Calgary

Xenbase (www.xenbase.org) supports biomedical, developmental and cell biology using *Xenopus*, the African and Western clawed frogs. Xenbase is the central repository for *Xenopus* genetics and genomics data and provides researchers with bioinformatic resources and tools for complex analysis. Our mission is to 1) provide the latest genomes linked to genes and orthologs; 2) curate published research/literature for disease models, experimental phenotypes, and gene expression; 3) annotate *Xenopus* genes with GO terms (molecular functions, biological processes and cellular components); and 4) collate diverse genomics data from high throughput sequencing in a central, searchable, database. *Xenopus* genomes, proteomes, genome annotation files and *Xenopus* gene-to-human gene ortholog mapping files are available from our download site. The backbone of *Xenopus* gene expression curation is the Xenopus Anatomy Ontology (XAO) and phenotype curation uses the Xenopus Phenotype Ontology (XPO), linking *Xenopus* disease models to Disease Ontology (DO) terms when appropriate. Xenbase also derives expression phenotype data from our standardized pipeline for processing high-throughput datasets from the NCBI Gene Expression Omnibus (GEO) repository. In addition, Xenbase has recently expanded our education resources including an anatomy atlas, normal tables of development, tables of anatomical staging landmarks, tables of key tissue and stage specific marker genes, high resolution images of developing and adult frogs, movies of embryonic development, and a set of open access illustrations of embryonic development. Aggregating all of this information in an easy to use and free to access web portal, Xenbase effectively connects *Xenopus* genes and phenotypes to human genes and diseases via multiple data resources including the Alliance of Genome Resources. Here we provide an overview of Xenbase resources, tools and curated *Xenopus* data, and data interconnectivity. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH).

595F **CRIS-cross: Efficient Generation of Combinatorial Genetic Variant Libraries** Han-Ying Jhuang, Gregory Lang Lehigh University

CRISPR-based genome engineering allows for precise and efficient editing of genetic sequences. However, the process also requires a significant amount of time, labor, and financial resources. To overcome this limitation, we use a modified yeast mating machinery that utilizes the natural efficiency of yeast mating but avoids the formation of hybrid nuclei. This system allows us to achieve high editing efficiencies and quickly generate combinatorial libraries of genetic variants. We are validating our system with known genetic interactions, and are creating a 3-way combinatorial library of genetic variants from evolution experiments. Our work has the potential to greatly increase our understanding of genetic interactions and provide insight into the evolution of species.

596F **Yeast Pathways at Saccharomyces Genome Database: Transitioning to Noctua & Alliance Pathways** Suzanne Aleksander[1], Dustin Ebert[2], Stacia R Engel[1], Rob Nash[1], Edith D Wong[1], Paul D Thomas[2], J. Michael Cherry[1], The SGD Project[1] [1]Genetics, Stanford University, [2]Department of Population and Public Health Sciences, University of Southern California

The *Saccharomyces* Genome Database (SGD, http://www.yeastgenome.org) is the leading knowledgebase for *Saccharomyces cerevisiae*. SGD collects, organizes and presents biological information about the genes and proteins of the budding yeast, including information concerning metabolism and associated biochemical pathways. The yeast biochemical pathways were originally sourced from the YeastCyc Pathway/Genome Database, which uses the metabolic pathways from MetaCyc. YeastCyc Pathways were imported into SGD in 2002, then SGD biocurators edited them as necessary to make them specific to *S. cerevisiae*, using publications from the primary literature. This comprehensive curation ensured that only the reaction directions and pathways that are physiologically relevant to *S. cerevisiae* were included, and also provided written summaries for each pathway. Loading, editing, and maintaining YeastPathways displayed at SGD was accomplished using the Pathway Tools software.

YeastPathways have now been available at SGD for over 20 years, with the last major content update in 2019. Recently, YeastPathways have been transitioned from a curation system that uses Pathway Tools software to one that uses the Gene Ontology curation platform Noctua, the interface SGD already uses to curate GO annotations. Each of the 220 existing pathways in YeastPathways has been converted from Pathway Tools BioPAX format into separate GO-Causal Activity Models (GO-CAMs), which are available as Turtle (.ttl) files. The GOCAM structured framework allows multiple GO annotations to be linked, which is ideal for a metabolic pathway. Although many of the steps are automated, manual intervention was required throughout the process to complete the GOCAMs and to verify inferences made by the conversion tools. Using the Noctua curation interface for biochemical pathways, without the need for external software, will streamline curation. Additionally, each model's metadata is accessible to any interested party. GO-CAMs can be deconstructed into standard GO annotations, making the pathway information accessible for enrichment studies and other applications. YeastPathways as GO-CAMs will also be available through the Gene Ontology's GO-CAM browser and the Alliance of Genome Resources, as well as any other resources that display GO-CAMs. This upgrade to the SGD's curation process will also make YeastPathways more transparent and compliant with FAIR guidelines and TRUST principles.

597F **Single-cell DNA Sequencing via *in situ* Genomic Amplification & Combinatorial Barcoding** Parker E Crossland, Kara Schmidlin, Leandra Brettner, Isabella Valli-Doherty, Kerry Geiler-Samerotte Arizona State University

Single-cell DNA sequencing (scDNA-seq) can identify genetic differences between individual cells in a population, which has broad applications in studying cell biology. For example, because scDNAseq preserves haplotypes, it enables studying the fitness of combinations of mutations instead of just individual mutations. Current scDNA-seq methods require the physical separation of cells, which can be done manually or using machinery. Both are time-consuming and costly. Thus, most scDNA-seq studies are limited to a few hundred cells. These constraints make it challenging to multiplex samples and perform high throughput experiments. We are developing a novel method for sequencing the DNA of single cells in heterogeneous populations by using cells as their own container for *in situ* amplification and barcoding reactions, thus eliminating the need to isolate individual cells. To uniquely identify DNA from each cell, we use combinatorial barcoding, which creates cell-specific barcodes by using a unique combination of non-unique nucleotide sequences. Combinatorial barcoding allows for the pooling of cells, making the method multiplexable and enabling the analysis of dozens of samples containing thousands of cells each. Our flexible method allows for targeted sequencing of a region of interest and whole genome sequencing. We aim to optimize the method for various organisms and applications to make it accessible to many research groups.

598F **Extending the GRNsight application for visualizing small-to-medium gene regulatory networks to incorporate physical protein-protein interaction data from SGD** Kam D Dahlquist[1], Onariaginosa O Igbinedion[2], Ahmad R Mersaghian[1], Ngan N Tran[2], Deepa V Dabir[1], John David N Dionisio[2] [1]Biology, Loyola Marymount Univ, [2]Computer Science, Loyola Marymount Univ

A gene regulatory network (GRN) consists of genes, transcription factors, and the regulatory connections between them which govern the level of expression of mRNA and protein from genes. GRNmap is an open source MATLAB program that performs parameter estimation and forward simulation of a differential equations model of a GRN based on time course gene expression data. GRNsight is an open source web application for visualizing small-to-medium scale GRNs, especially models produced by GRNmap. GRNsight reads the Excel input and output workbooks from GRNmap and automatically displays the model data as a graph with colored nodes (expression data) and edges (estimated regulatory weights). We recently implemented a backend PostgreSQL database for GRNsight, populated with five public gene expression datasets and regulatory network data from the *Saccharomyces* Genome Database (SGD). A user can select genes to include in the GRN, and GRNsight will automatically layout the network using regulatory connections from SGD. The user can then select one of the time course gene expression datasets with which to color the nodes and export to a properly-formatted GRNmap input workbook. GRNmap can then be used to estimate the regulatory weights (activation vs. repression and magnitude of the relationship). Although GRNsight was originally designed for GRNs, it has the general applicability for displaying any appropriately-sized, weighted or unweighted network with directed edges. Previously, GRNsight was limited to only displaying graphs with directed edges and was unable to display protein-protein physical interaction (PPI) networks, as they are represented by undirected graphs. Thus a protein-protein physical interaction network mode was created to visualize these models in GRNsight. Additionally, a backend database

was added to store PPI data obtained from SGD, so that users can generate a PPI network within GRNsight the same way they can generate a GRN. The graphical user interface was updated to be able to toggle between these two graph modes, enabling comparison of these two types of data. GRNmap is available at http://kdahlquist.github.io/GRNmap/; GRNsight is available at http://dondi.github.io/GRNsight.

599F **Generating a transgenic fish for in vivo cell cycle studies in glial cells** Jenny R Lenkowski[1], Cas A Sturdivant[2], Edna A Ferreira[1] [1]Goucher College, [2]Biological Sciences, Goucher College

Glial cells in the central nervous system provide a variety of functions including homeostatic support of neurons and synapse regulation as well as acting as stem cells. Glia develop from the same neuroepithelia as neurons and in some animals can be reactivated to act as tissue-specific stem cells to regenerate destroyed neurons, an area of research in our lab. Therefore, we are developing a system to study cell cycle dynamics in glial cells in the zebrafish model system. In the zebrafish fluorescent ubiquitination-based cell cycle indicator (zFUCCI) system, the fluorescent protein mCherry is tagged with a fragment of chromatin licensing and DNA replication factor 1 (CDT1) and visible during G1 phase, while mAzamiGreen (mAG) is tagged with a fragment of geminin and visible S through M phase. We are generating a *gfap:zFUCCI* system with the glial fibrillary acidic protein (gfap) promoter, thus, *gfap:mCherry_Cdt1* and *gfap:mAG_Gem* constructs will only be expressed in glia. In the neural retina, this would allow Müller glia to be observed via fluorescent imaging after a neuronal lesion when they reenter the cell cycle and cell cycle dynamics quantified when genes or pathways are manipulated. We have confirmed the zFUCCI construct sequences by culturing *E. coli* carrying the construct to sequence and injected linearized constructs into fertilized zebrafish eggs to establish transgenic lines. These *gfap:zFUCCI* lines will be used to then study how cell signaling pathways regulate cell cycle dynamics during development and regeneration in the nervous system.

600F **Integrative 3D Zebrafish Microanatomical Atlas** Khai C Ang[1,2], Jean E Copper[1], Mee S Ngu[1], Daniel J Vanselow[1], Keith C Cheng[1] [1]Pathology, Penn State College of Medicine, [2]Penn State Functional Genomics Core, Penn State College of Medicine

The zebrafish is a vertebrate model organism with significant genetic, cellular, and physiological similarities with humans. Its powerful genetics and advanced imaging facilitate the elucidation of genetic functions, embryonic development, and disease mechanisms. Traditional 2D histology offers cellular and subcellular detail across cell types and is the cornerstone of histopathological diagnosis. Its inherent limitations include its two-dimensional perspective, under-sampling, and section-based artifact, which lead to inadequate understanding of the three-dimensional structure of cells (including volume, shape, texture) and spatial relationships between cells in different tissues and disease states. To enable the computational phenotyping of whole organisms without sampling bias, we developed a 3D form of histology, *histotomography*, that features centimeter field-of-view, and submicron voxel resolution. This enables volumetric measurements of cells and the relationships between cells and tissue structures in the context of the whole organism.

The 2D histology component of existing atlases includes high-resolution images of the organisms' cells, tissues, and organs. We propose to add a microCT component to atlases that will add 3D representations of cells and organs, allowing researchers to interrogate the spatial relationships between the structures and exploration of microanatomy in virtual environments. Integrative atlas resources will allow access to multi-omic data in the context of the whole organism. MicroCT images will serve an integrative role, anchoring histology, fluorescence and ultrastructural imaging, and large-scale "-omic" data across length scales. Integrating "-omics" data elevates atlases to dynamic anatomical guides for molecular data in the context of the whole organism platform to elucidate how genetic variation and external perturbations cascade through multiple biological scales to influence phenotype.

The tools of the 3D zebrafish atlas resource are being created as a foundation for future application across model organisms and human atlases to anchor research and educational advances in our understanding of biology, disease, and development.

601F **Improving toxicology data compatibility within and across species and advancing a community-led zebrafish toxicology phenotype atlas.** Anne E. Thessen[1], Melissa Haendel[1], Alexa Burger[2], Sabrina Toro[1] [1]Department of Biomedical Informatics, University of Colorado, Anschutz, [2]Department of Pediatrics, Section of Developmental Biology, University of Colorado, Anschutz

Zebrafish have greatly advanced toxicology and environmental studies as a valuable animal model because they are easy to manipulate, breed, and observe during development. However, the absence of universal standards for toxicology data significantly impedes scientific progress and therefore human and environmental health.

The Zebrafish Toxicology Phenotype Atlas is a new, community-built standard for annotating zebrafish toxicological exposure and their phenotypic outcomes (toxicophenotypes). We are designing a toxicophenotype data model that enables comprehensive representation of toxicological exposures, including time course, toxicants, and concentrations; genotype and

genetic manipulations; phenotypic endpoints. This data model is developed with the community and leverages a variety of different toxicological use cases, from multiple species. Ensuring that the core of this data model is applicable across organisms will allow data integration and interoperability across species and across toxicological studies.

We will create a toxicophenotype annotation toolkit that will allow users to annotate their data based on the data model, and will instantiate a zebrafish toxicophenotype atlas web application. This atlas will serve as a visual definition of the standards and their documentation for examining variations of specific phenotypes by laboratories in the community. Users will be able to explore and query exposures and phenotypes of interest and see example images demonstrating the phenotypes.

This project is governed and driven by the community, which includes diverse stakeholders in toxicology and environmental health sciences, ensuring fit-for-purpose design and sustainability.

Realizing interoperable toxicophenotypic data is crucial to improving data integration across scale and granularity, and across species; thereby accelerating our understanding of environmental influences on human health.

602S **Assessing behaviour across organisms through patterns in multi-variate data** Christopher James[1,2], Christopher James[3] [1]EMbody Biosignals Ltd., [2]Biomedical Engineering Institute, University of Warwick, [3]EMbody Biosignals LTD

Whether its tracking human behaviour through activity monitoring or nematode behaviour on a dish, AI and machine learning based data analysis solutions provide a powerful way to undertake real-time monitoring to gain insight on trends, history and prediction, as well as to detect problems quickly.

Of the various methods in the literature a) Rules based systems take time and extensive knowledge of the end user and b) Statistical approaches are fast but rely on one-size fits all techniques. Machine learning based methods remove these limitations by automatically learning behaviour patterns for each individual organism. Crucially, these methods provide a data source agnostic approach that can work on any numeric, categorical or event based data. For example, through the use of a video microscope, longitudinal patterns in C.elegans can be captured and examined over very long periods of time resulting in learned generative models to predict behaviour in both individual as well as population models. The same methods can be used to assess multi-sensor scenarios in more complex organisms yet with similar, detailed measures of complex behaviour as outcomes.

603S **High-Efficiency Integration of Large DNA Fragments by Cas9 Cleavage and Single-Strand Annealing in *Drosophila*** Jeff Sekelsky, Susan McMahan Biology, University of North Carolina

Numerous types of genome editing with CRISPR/Cas9 have been described. One goal that remains difficult is integration of large DNA fragments, as this challenges the processivity of homology-directed repair (HDR). The single-strand annealing (SSA) repair pathway should allow pasting in of large fragments without the need for a copying step as in typical HDR. When a DNA double-strand break (DSB) is made, the first step in HDR is resection of the ends to generate long 3’-ended single-strand overhangs. If the DSB is between direct repeats, this resection exposes complementary sequences that are efficiently annealed. In this chromosomal SSA the result is collapse to a single repeat with loss of intervening sequences.

In our approach, a payload to be integrated into the genome is flanked by homology arms (HAs) that match sequences adjacent to the genomic target site(s). The [HAleft] + payload + [HA right] unit is cut out of the plasmid when the genomic target site is cut. Resection of both the chromosomal site and the linear fragment released from the plasmid exposes complementarity between the HAs and the target site, promoting SSA to integrates the payload. This approach was developed independently by Oguz Kanca to integrate smaller fragments into thousands of Drosophila genes (PMC9239680).

We built a vector that allows (1) introduction of one or more gRNA sequences for targeting the genome (these are added downstream of the gRNA to cut the vector, which is already present); (2) simultaneous cloning of both HAs; and (3) sites for the payload between the HAs. Each step is done by highly efficient GoldenGate cloning. For the payload step the vector can function as a GoldenBraid omega vector to allow assembly of two or more pieces from plasmids or fragments. Additional vectors we’ve made include one with chromatin insulators, versions with eGFP or mCherry as payloads (just add gRNA and HAs), and variants that have additional gRNA sites for downstream engineering in the fly.

We tested our strategy by targeting a site in ebony. The vector carried a 12.5-kb payload including eGFP and used ~400-bp HAs. Among G0 flies after injection, 18 of 29 (62%) had ebony progeny, and 51% of ebony flies (27% of all progeny) expressed eGFP. We are now testing larger payloads and precise editing by replacing a genomic region after two cuts. We’ve also domesticated many parts (Drosophila promoters, genes, recombination target sites, etc.) that can be used in GoldenBraid cloning.

604S **New from the TRiP: large scale resources for gene perturbation, gene expression, and protein detection** Jonathan

Zirin[1], Ben Ewen-Campen[1], Justin A Bosch[1], Ah-Ram Kim[1], Raphael Lopes[1], Neha Joshi[1], Barbara Jusiak[2], Lu-ping Liu[1], Christians Villalta[1], Alexandria Risbeck[1], Elizabeth Filine[1], Yousuf Hashmi[1], Corey Forman[1], Aram Comjean[1], Yanhui Hu[1], Stephanie Mohr[1], Norbert Perrimon[1,3] [1]Genetics, Harvard Medical School, [2]Physiology and Biophysics, University of California, Irvine, [3]Howard Hughes Medical Institute

The Transgenic RNAi Project (TRiP) is an in vivo functional genetics platform that has generated >20,000 shRNA and sgRNA fly stocks for the research community. These resources, distributed to the community by the BDSC, provide powerful, versatile, and transformative tools for gene knockdown, knockout, and activation. Researchers can now easily access fly stocks useful to "dial down" or "dial up" genes covered by the collection, in a variety of developmental stages and tissues. Here we describe new in vivo resources designed to enable: 1) combinatorial studies using multiple binary expression systems. Focusing on genes with well-characterized GAL4 expression patterns, we generated a set of more than 40 LexA-GAD and QF2 insertions by CRISPR knock-in and verified their tissue-specificity in larvae; 2) validation of scRNA gene clusters using split-Gal4 lines. Using an algorithm that determines the smallest unique set of marker genes that define a cluster, we are generating and validating several hundred highly specific Gal80-repressible split-Gal4 knock-in lines; 3) detection of fly proteins. We are inserting a NanoTag epitope at the C-terminus of more than 300 high confidence Drosophila orthologs of human mitochondrial disease genes, which can then be recognized by an existing high-affinity nanobody against the NanoTag. These new resources will dramatically expand the scope of available reagents for gene expression and detection of fly proteins.

605S **Optogenetics for All: NinaB Cleavage of *Beta*-Carotene as a Source of *all-trans* Retinal** Karen L Hibbard[1], Ilya Vilinsky[2], Thomas A Ravenscroft[1], Glenn Turner[1], Yoshi Aso[1] [1]HHMI Janelia Research Campus, [2]University of Cincinnati

Optogenetics has become a valuable tool for investigating memory, learning, and behaviours in *Drosophila* and many other species. It also has become an exciting science educational tool that has been successfully implemented in elementary and secondary schools. There are many different variants of opsins: ion channels (channelrhodopsin), pumps (halorhodopsins), or coupled G-Proteins (visual opsins). All opsins share a similar structure of 7 transmembrane domains and bind a retinal molecule (chromophore) within the 7th transmembrane domain. The retinal chromophore absorbs a photon of light and rapidly isomerize inducing a structural change in the protein. *Drosophila* are unable to synthesize retinal and it must be derived from carotenoids in their diet. In the majority of channelrhodopsin variants, the amounts of carotenoids obtained from standard cornmeal fly food is insufficient to supply both the visual system and the transgenic channelrhodopsins. Fly food is usually supplemented for optogenetic experiments in the range of 0.2 -0.4 uM of *all-trans*-retinal which is costly. NinaB, a *beta, beta*-carotene-15,15'-oxygenase, cleaves carotenoids into two retinal molecules. Confocal imaging of *ninaB-GAL4/UAS-GFP*, identified glial cells as the putative site of *ninaB* expression. We are investigating the efficacy of pan glial over-expressed NinaB cleaved *beta*-carotene as the source of *all-trans* retinal for optogenetic experiments. Current experiments are comparing the cost-effective *beta*-carotene supplementation versus *all-trans* retinal in optogenetic behaviors and electrophysiology (e.g., Moonwalker behavior, learning within the mushroom body, and excitatory junction potentials at the larval neuromuscular junctions).

606S **Selective depletion of histone transcripts to interrogate histone residue function** Oscar M Arroyo, Daniel J McKay University of North Carolina - Chapel Hill

At the earliest stages of metazoan development, a single diploid nucleus begins dividing and eventually creates a diversity of cell types with specialized functions in the adult. No genetic information is gained or lost during development; rather, epigenetic mechanisms are responsible for maintenance of gene expression programs underlying cell identity. Post-translational modifications on histones, the main protein component of chromatin, are a major source of epigenetic information. Despite their importance, direct examination of histone function in metazoans is challenging due to their high copy number and location on multiple chromosomes. *Drosophila melanogaster* provides an excellent experimental system for studying histones in epigenetic gene regulation as all 100 copies of the canonical histone genes exist at a single locus in a tandem array. Homozygous deletion of the histone gene locus is lethal but can be rescued by a transgene carrying 12 copies of the wild-type histone repeat unit. By mutating the transgenic histone genes to encode target residues that cannot be post-translationally modified, we have engineered mutant genotypes to directly test histone residue function. Although this "histone replacement platform" has expanded our understanding of epigenetic gene regulation, the scope of experimental approaches that can be employed remains limited because many histone mutations are lethal and can only be studied by generating small clonal populations of mutant cells. Here, we present an approach that further expands our ability to interrogate histone function by improving spatiotemporal control over histone depletion. Utilizing the GAL4/UAS system to express transgenic siRNAs targeting the 3'UTR of the *D. melanogaster* histone H3, we can selectively deplete endogenous histone gene expression in a targeted manner. We find that H3 depletion results in a severe proliferation defect. However, we can rescue this defect by providing transgenic H3 bearing the 3'UTR from the *Drosophila simulans* H3 gene, which renders it insensitive to siRNA-mediated knockdown. Coupling H3 depletion with a knockdown-insensitive mutant transgene allows us

to generate entire tissues expressing mutant histones, thus expanding the scope of experimental approaches beyond those employed in small populations of cells. We propose to use this genetic platform to generate fully mutant tissues or embryos to further interrogate the requirement of specific histone residues.

607S **Repurposing synthesized homology donor intermediates to generate novel genetic reagents** Oguz Kanca[1,2], Catherine Grace Burns[1,2], Megan Cooper[1,2], Ali Hosseini Bereshneh[1,2], Wen-wen Lin[1,2], Liwen Ma[1,2], Ming Ge[1,2], Robert W. Levis[3], Hugo J. Bellen[1,2,4] [1]M & H Genetics, Baylor College of Medicine, [2]Duncan Neurological Research Institute, Texas Children Hospital, [3]Department of Embryology, Carnegie Institution for Science, [4]Department of Neuroscience, Baylor College of Medicine

The Gene Disruption Project (GDP) aims to generate versatile reagents to facilitate in depth functional characterization of fly genes with an emphasis on fly genes that are conserved in humans. The GDP has generated thousands of alleles with transposable elements and more recently CRISPR-mediated homologous recombination (CRIMIC). We have developed streamlined methodologies to accomplish CRIMIC transgenesis to make targeting thousands of genes feasible. Our current methodology uses commercial DNA synthesis to generate a homology donor intermediate vector which contains sgRNA coding sequences that both guide the cutting of the target locus and the linearization of the circular homology donor construct *in vivo* in the Cas9-expressing germline. It contains short homology arms that enable homologous recombination. This construct design allows single step directional cloning of any payload in the homology donor intermediate vector to generate a donor construct containing all the components required for successful homologous recombination. To date, we have generated over 3000 transgenic alleles that express GAL4 in the expression pattern and dynamics of the targeted gene. These transgenes not only generate strong loss of function alleles but also are useful to determine the expression pattern of the targeted gene by using the GAL4 to drive the expression of a UAS-RFP reporter. Furthermore, the GAL4 can be used to drive a cDNA of the human orthologue of the target gene to test whether the human orthologue can rescue the fly mutant phenotype. Recently, we started to repurpose the synthesized homology donor intermediate vectors to generate GFP protein trap alleles for over 80 genes. These GFP alleles are very versatile and allow detection of the subcellular localization of the targeted gene product, immunopurification of the gene product and its interactors using anti-GFP antibodies. The GFP tag allows removal of the gene product by using genetic tools such as DegradFP or iGFPi. Repurposing the homology donor intermediates offers many advantages compared to RMCE based approaches. First, the homology donor intermediates contain all the components required for homologous recombination. Second, it allows using the same injection stock to create different reagents. Third, if the CRIMIC allele is in an essential gene, only half of the injected embryos are viable whereas injection in wildtype stocks do not suffer from this effect. We are also currently testing whether it is possible to replace the target gene with human cDNA directly at the endogenous locus. Replacing the fly gene with human cDNA directly in the locus will allow more precise matching of expression levels, which may increase the success rate of functional replacement of the fly gene with the human gene. Hence, repurposing the homology donor intermediates opens new avenues to easily develop novel applications to manipulate fly genes.

608S **Higher-resolution pooled genome-wide CRISPR knockout screening in insect cell lines using integration and anti-CRISPR (IntAC)** Raghuvir Viswanatha[1], Samuel Entwisle[1], Claire Hu[1], Stephanie E Mohr[1], Norbert Perrimon[1,1,2] [1]Genetics, Harvard Medical School, [2]Howard Hughes Medical Institute

CRISPR screens provide a systematic, scalable approach for linking genotype to phenotype. We have successfully conduct CRISPR screens in insect cells (*Drosophila* or *Anopheles*) using plasmid transfection and site-specific integration to introduce sgRNA libraries, followed by PCR and sequencing of integrated sgRNAs at the end of the assay. Although the method is highly effective, quantitative analysis revealed room for improvement. With our approach, constitutive Cas9 activity is important. However, having Cas9 activity present early in the screen workflow could be problematic, because immediately after library transfection many active sgRNA expression plasmids are present in cells but only one or a few of sgRNAs will become integrated, creating the potential for a mismatch between genome edits that result in a phenotype and the sgRNA sequence detected. To avoid this, we previously used a weak promoter (*DmU62*) for sgRNAs, which likely delayed activity, favoring recovery of edits attributable to integrated sgRNAs but also reducing efficiency. Here, we asked if suppressing Cas9 activity in the early post-transfection period would allow us to combine constitutive Cas9 expression with use of a stronger promoter for sgRNAs for improved screen quality. To do this, we co-transfected a plasmid expressing the anti-CRISPR protein AcrIIa4 at the time we introduced the sgRNA library, now driven by the higher-strength *DmU63* promoter, and the integrase that mediates integration of the sgRNA. Early on, we expect that sgRNAs are expressed but Cas9 activity is suppressed by anti-CRISPR. Later, when the anti-CRISPR plasmid has been lost, Cas9 acts together with the integrated sgRNA to edit the target gene. This approach, which we call “intAC” for integrase with anti-CRISPR, successfully delayed CRISPR activity and, when tested in combination with a new machine learning-based genome-wide sgRNA library, dramatically increased the number of high-confidence fitness genes detected in a ‘drop-out’ screen. This dataset provides the most complete set of cell fitness genes yet assembled for *Drosophila*. We also used the intAC approach together with a targeted sgRNA sub-library for positive selection

of nucleoside transporters. Overall, we demonstrate that the introduction of anti-CRISPR along with sgRNAs outperforms existing virus-free pooled CRISPR screening strategies.

609S **Drosophila Community Strains Pangenome: A Pangenome Resource Built From Highly Contiguous Genome Assemblies Of *Drosophila melanogaster* Community Strains** Mahul Chakraborty Department of Biology, Texas A&M University

Understanding the genotype-to-phenotype relationship in model organisms relies on community strains that various laboratories have developed. These strains often carry mutations and transgenes for genetic mapping and reverse genetics experiments, such as RNAi and CRISPR. However, in most cases, the genetic background of these strains is unknown. When interpreting the functional consequences of genetic perturbations in these strains, we assume that the study strains share a “more-or-less” same genome as the community reference genome. I have recently shown that the presence of genome structural variants, such as copy number changes, deletions or insertions, insertion of transposable elements, and inversions in widely used *Drosophila melanogaster* community strains can severely mislead our understanding of genotype-to-phenotype relationships. The ideal solution to this problem would be to build a reference genome of all laboratory strains used by the members of a model organism community. However, sequencing the genomes of all laboratory strains in *D. melanogaster* is unnecessary because the genetic background of many strains are derived from a few strains. A pangenome of these strains would be sufficient for the majority of experiments investigating the sequence-to-phenotype relationship. I initiated the Drosophila Community Strains Pangenome (DCSP) project to build such a resource. It is a publicly available pangenome constructed with highly contiguous, reference-quality genome assemblies of commonly used *D. melanogaster* strains, including wild-type and transgenic strains. The genome assemblies are built with Pacific Biosciences HiFi reads, which ensures high sequence accuracy across the assembled genome, including the repetitive sequences. The pangenome can be downloaded and searched using alignment tools. The initial release of DCSP features the genomes of 12 popular *D. melanogaster* strains, including the genetic background of commonly used CRISPR and RNAi stocks, with plans for further expansion based on community feedback. Annotation of comprehensive structural variants in these genomes revealed several variants with potential phenotypic effects that can confound the interpretation of functional experiments involving these strains or their genetic background. The DCSP project will be a valuable resource for *Drosophila* researchers, enabling accurate interpretation of results and advancing our understanding of genotype-to-phenotype relationships.

610S **Characterization of shock wave effects using nanoparticles in syncytial embryos of *Drosophila melanogaster*** Daniel Tapia Merino, Achim Max Loske Mehling, Pedro Salas Castillo, Juan Rafael Riesgo Escovar Universidad Nacional Autonoma de Mexico

In this work, nanoparticles have been used as fiducial markers to evidence the insertion of exogenous material in the early embryo of *Drosophila melanogaster*, which is a syncytium. The aim is to achieve the insertion of nanoparticles through the application of underwater shock waves, a method that has been used successfully to create pores in other cell membranes and cell walls, such as those of fungal cells. In our shock wave protocol, the majority of embryos treated with shock waves in a suspension of nanoparticles survived to adulthood. We used carbon quantum dots and gold nanoparticles to evaluate whether they can be seen in sections of *Drosophila* larva by microscopy (fluorescence and electron transmission), after treatment with shock waves. In a complementary test, larva showed transient expression with a plasmid encoding GFP after underwater shock wave treatment when they were syncytial early embryos. The demonstration that exogenous material can penetrate the outer layers (chorion and vitelline membranes) as well as the cellular membrane of the early *Drosophila* embryo applying this regime, may open the door to diverse biological applications.

611S **Single-embryo ‘omics to monitor gene expression and metabolite changes in a sex-specific manner during early *Drosophila* development** J. Eduardo Pérez-Mojica[1], Zachary Madaj[2], Christine Isaguirre[3], Kin Lau[2], Joe Roy[1], Krittika Sudhakar[1], Ryan Sheldon[3], Adelheid Lempradl[1] [1]Department of Metabolism and Nutritional Programming, Van Andel Institute, [2]Bioinformatics and Biostatistics Core, Van Andel Institute, [3]Mass Spectrometry Core, Van Andel Institute

Early embryonic development lays the foundation for embryo structure and cellular differentiation. While the underlying transcriptional events have been widely studied, little is known about the concurrent metabolic processes. To study early embryo metabolism and understand its relationship with gene expression, we developed a method to simultaneously measure RNA and metabolites from individual embryos in *Drosophila melanogaster*. This method utilizes single-embryo RNA-sequencing data to establish a high-resolution developmental trajectory (pseudo-time). Single-embryo metabolomics data are then matched, enabling continuous metabolite analysis during early embryonic development. The resulting dataset provides a detailed picture of early embryo development up to the onset of gastrulation (≤3 hours) in *Drosophila*. The high resolution and sensitivity of this method allows for the identification of subtle changes in both transcripts and metabolites. As an example, our data show that deoxyribonucleotide metabolism is highly dynamic, showing a marked and rapid decreased in deoxyribonucleoside triphosphates at the onset of the zygote genome activation. Due to a high-resolution data, we identified novel transcripts with significantly different expression between males and females. Together, our method provides a new tool

with higher sensitivity to investigate the metabolism-transcription axis in the embryo. Beyond normal embryo development, this method can be used to characterize the role of developmental/metabolic genes during early embryonic development and investigate the mechanisms underlying embryo re-programming by maternal exposures.

612S **Upgraded genome browsers at the Rat Genome Database support comparative and translational studies** Jennifer Smith[1], Stanley JF Laulederkind[1], G Thomas Hayman[1], Shur-Jen Wang[1], Monika Tutaj[1], Mary L Kaldunski[1], Mahima Vedi[1], Wendy M Demos[1], Marek A Tutaj[1], Jyothi Thota[1], Logan Lamers[1], Adam C Gibson[1], Akhilanand Kundurthi[1], Varun Reddy Gollapally[1], Kent C Brodie[2], Stacy Zacher[3], Jeffrey L De Pons[1], Melinda R Dwinell[1], Anne E Kwitek[1] [1]Physiology, Medical College of Wisconsin, [2]Clinical and Translational Science Institute, Medical College of Wisconsin, [3]Finance and Administration, Medical College of Wisconsin

The Rat Genome Database (RGD, https://rgd.mcw.edu), a cross-species knowledgebase and the premier online resource for rat genetic and physiologic data, has recently added two advanced comparative genome browsers to our suite of innovative analysis tools--JBrowse 2 and VCMap. JBrowse 2 (https://rgd.mcw.edu/jbrowse2/) is a newly developed genome browser with improved functionality for structural variant and comparative genomics visualization. JBrowse 2 shares many features with its predecessors GBrowse and JBrowse 1, such as the ability to select and view multiple data types in a single view, easy zooming and navigation across a chromosome, and the ability to search for the name or ID of a genome feature, for example a gene or QTL, and go directly to that region of the genome. In addition, it provides expanded functionality designed to facilitate comparative studies, including the linear synteny viewer for cross-species analyses, the breakpoint split view and circular plot for structural variants, and a dot plot viewer for whole genome alignments.

JBrowse 2 browsers have been set up for all ten RGD species, including browsers for multiple assemblies for each species where those are available. For rat in particular, a substantial set of tracks is available. In addition to gene, QTL and strain tracks, RGD's JBrowse 2 provides tracks for RNA-Seq BAM alignments, strain-specific variants, and ATAC-Seq and ChIP-Seq epigenetics data aligned onto the mRatBN7.2 assembly. Tracks for consolidated variants from the European Variant Archive are available for all applicable species, including rat, mouse, dog, pig and green monkey, allowing comparisons with human variants from ClinVar and the GWAS Catalog for a gene or region of interest.

For those interested in synteny across more than two species, RGD is developing the Virtual Comparative Map tool (VCMap, https://rgd.mcw.edu/vcmap/). VCMap, currently released as a beta version, provides a bird's eye view of synteny between two or more species/assemblies, whereas the synteny viewer in JBrowse 2 is limited to only two. Since the original version of VCMap, substantial improvements have been made to the performance and navigation for comparisons of syntenic regions across species. In addition to genes, tracks for data such as genomic variant densities can be added to the display. In conclusion, RGD's JBrowse 2 and VCMap provide valuable functionality for researchers engaging in comparative genomics and translational medicine.

613S **Pseudorabies virus upregulates low-density lipoprotein receptors to facilitate viral entry** ming shengli Henan Agricultural University

Pseudorabies virus (PRV) is the causative agent of Aujeszky's disease in pigs. The low-density lipoprotein receptor (LDLR) is a transcriptional target of the sterol-regulatory element-binding proteins (SREBPs) and participates in the uptake of LDL-derived cholesterol. However, the involvement of LDLR in PRV infection has not been well characterized. We observed an increased expression level of LDLR mRNA in PRV-infected 3D4/21, PK-15, HeLa, RAW264.7, and L929 cells. The LDLR protein level was also upregulated by PRV infection in PK-15 cells and in murine lung and brain. The treatment of cells with the SREBP inhibitor, fatostatin, or with SREBP2-specific small interfering RNA prevented the PRV-induced upregulation of LDLR expression as well as viral protein expression and progeny virus production. This suggested that PRV activated SREBPs to induce LDLR expression. Furthermore, interference in LDLR expression affected PRV proliferation, while LDLR overexpression promoted it. This indicated that LDLR was involved in PRV infection. The study also demonstrated that LDLR participated in PRV invasions. The overexpression of LDLR or inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9), which binds to LDLR and targets it for lysosomal degradation, significantly enhanced PRV attachment and entry. Mechanistically, LDLR interacted with PRV on the plasma membrane, and pretreatment of cells with LDLR antibodies was able to neutralize viral entry. An in vivo study indicated that the treatment of mice with the PCSK9 inhibitor SBC-115076 promoted PRV proliferation. The data from the study indicate that PRV hijacks LDLR for viral entry through the activation of SREBPs.

614S **RNA-Seq Technologies Identify Causal Mutations in Mice with Mendelian Disorders** David Bergstrom[1], Son Yong Karst[1], Belinda S Harris[1], Melissa L Berry[1], Whitney Martin[1], Heather E Fairfield[2], Narayanan Raghupathy[3], Laura G Reinholdt[1] [1]The Jackson Laboratory, [2]MaineHealth Institute for Research, [3]Bristol Myers Squibb

To investigate disease manifestation seen among patients and across mouse strains with Mendelian disorders, the identification of causal mutations is fundamental. Within The Jackson Laboratory's Comparative Mendelian Disease Genomics (CMDG) program (R24 OD021325/OD/NIH HHS/United States), we are exploring alternative technologies to identify disease-causative mutations where whole-exome sequencing alone has been insufficient. One such technology is transcriptome sequencing (RNA-Seq). Here, we report the analysis of sequenced transcriptomes from disease-relevant tissues for forty-seven (47) mouse models across a broad spectrum of Mendelian disorders (*e.g.*, those affecting craniofacial development, metabolism, neurological function, pigmentation, skeletal morphogenesis, *etc*.). Our analyses include the detection of single-nucleotide polymorphisms (SNPs) and an analysis of differential (gene) expression (DE), as well as an interrogation of changes among transcript isoforms. In many instances, confirmation of causation has been provided through CRISPR modeling and complementation testing. Our analyses highlight many instances where transcriptome sequencing has succeeded in identifying disease-causative mutations where other technologies have not, and suggests a further role for RNA-Seq, not only for mutation discovery in model organisms, but also in the diagnosis of Mendelian disease among patient populations.

615S **Can AI Outperform Well-Established Antimicrobial Resistance Gene Databases such as ResFinder and CARD?** Kristiene Recto, Pleuni S. Pennings Biology, San Francisco State University

The rise in antimicrobial resistance genes among microbes is a significant global health threat. This rise means that the antibiotics we use to treat infections become less effective, making it harder to fight these harmful microorganisms.

To combat infections and customize effective treatments, it's essential to know whether an infection is resistant to a certain drug. Machine learning combined with genomic data is a promising tool for proving valuable insight in this field. While machine learning has demonstrated efficient predictions in drug resistance, achieving the desired higher accuracy values remains a challenge. E coli is a species with a lot of diversity and can be divided into so-called phylogroups (A, B1, B2, C, D, E and F). It's currently unclear whether phylogroups affect prediction accuracies. In this study, we aim to bridge this knowledge gap by investigating the relationship between phylogroups and ML prediction accuracies in hopes of improving the ability to combat drug-resistant infections.

This study utilizes an openly accessible dataset of E. coli genomes from the UK to evaluate the prediction accuracies of the Gradient Boosted Tree Machine Learning model against ResFinder and Comprehensive Antibiotic Resistance Database (CARD). Gradient Boosted Tree is a branch of machine learning that combines predictions of multiple decision trees, with each tree correcting the errors of the previous tree to improve overall predictive accuracy. ResFinder is a database identifying acquired genes and finding chromosomal mutations mediating antimicrobial resistance in bacteria's total or partial DNA sequences. CARD offers analysis tools for molecular sequences, such as BLAST and the Resistance Gene Identifier (RGI) software, which utilize homology and SNP models to predict the resistome. In predicting the accuracies of antibiotic resistance profiles in E. coli, we will compare each model with five distinct E. coli Phylogroups (A, B1, B2, D and F) and apply our method to six antibiotic drugs. For each combination of drug and phylogroup, we will determine whether machine learning or the traditional CARD/Resfinder approach yield better results. Our project is dedicated to enhancing machine learning methods for quickly and accurately predicting antibiotic resistance. This advancement holds the potential to improve patient health outcomes significantly.

617S **Empowering disease knowledge representation with the Disease Ontology Knowledge (DO-KB)** J. Allen Baron, Claudia M. Sanchez-Beato Johnson, Lynn M. Schriml University of Maryland School of Medicine

The Human Disease Ontology (DO) is a FAIR genomic resource fostering cross-domain disease data integration and analysis, at model organism databases (e.g., FlyBase, MGD, RGD, SGD, WormBase, Xenbase, ZFIN) and by the broader biomedical community. It provides expertly curated, human-readable, and machine-actionable disease data with a comprehensive etiological disease classification spanning genetic, infectious, cancer, environmental, complex, rare, and common diseases. It links across authoritative biomedical resources with over 37,000 clinical vocabulary cross mappings and semantically represents diseases with mechanistic drivers and characteristics like mode of inheritance, phenotypes, chemical and environmental exposure, anatomy, and infectious agents.

The DO is a collaborative project that assists with disease data standardization and supports a growing disease data ecosystem, having been incorporated into over 390 biomedical resources and used in thousands of individual research projects since its inception in 2003. To expand this ecosystem and help researchers actionably leverage the DO in their research, genomic and machine learning applications and tools at disease-ontology.org that aid in exploration, access, connection, and search of disease data are highlighted. These tools include the Disease Ontology browser and new Disease Ontology Knowledgebase (DO-KB) SPARQL service and faceted search interface. The DO-KB SPARQL service empowers data discovery, extraction, and connection to diverse data across the web through the addition of DO-specific and federated query options, while the DO-KB

faceted search interface simplifies and enhances accessibility to the disease-defining semantic data in the DO, eliminating the need for users to have knowledge of programming and semantic logic to explore disease relationships.

Disease knowledge within the Disease Ontology and the disease data ecosystem it supports is continually updated and expanding. Greater collaboration will foster FAIR-er disease data access and open new avenues in biomedical research.

618S **Employing tRNA Mutations for Enhanced Non-canonical Amino Acid Incorporation** Berenice Guerra[1], Evan Kalb[2] [1]Health and Sciences School, Universidad del Sagrado Corazón, [2]University of Minnesota

Transfer RNAs (tRNAs) are essential for protein synthesis by delivering amino acids to the ribosome. Wild-type translation favors L-**α**-amino acids because of the specificity of aminoacyl-tRNA synthetases (AARs), EF-Tu binding, and ribosomal interactions. Tryptophanyl-tRNA synthetase has been observed to aminoacylate both D-tryptophan and *N*-**α**-methyl-tryptophan onto native $tRNA^{trp}$, but these substrates are unable to be incorporated in translation. Our hypothesis suggests that this incompatibility is due to reduced binding to EF- Tu and delivery to the ribosome. To overcome this, we introduced mutations in the T-stem of a native E. coli $tRNA^{Trp}$ and replaced it with the high affinity T- stem of $tRNA^{Asp}$. We overexpressed and purified the $tRNA^{TrpAsp}$ mutant using a hybridization probe purification scheme and confirmed its purity using a combination of Urea-PAGE and MALDI-TOF mass spectrometry. We found the overexpressed tRNA was hypomodified compared to wild type $tRNA^{Trp}$. Further, MALDI-TOF suggests $tRNA^{TrpAsp}$ is charged with L-Tryptophan. Our results suggest that wild type tRNAs can be mutated for the increased incorporation of noncanonical amino acids in native translation.

619S **Genome-wide CRISPR screening in a non-model Anopheles mosquito cell line** Enzo Mameli[1], Raghuvir Viswanatha[1], Claire Y Hu[1], William P Robins[2], John J Mekalanos[2], Stephanie E Mohr[1], Norbert Perrimon[1,3] [1]Genetics, Harvard Medical School, [2]Department of Microbiology and Immunobiology, Harvard Medical School, [3]Genetics, Howard Hughes Medical Institute

CRISPR screens are unbiased, systematic, linearly scalable approaches for linking genotype to phenotype. These screens had previously been restricted to mammals due to challenges in high-throughput delivery of a sgRNA library into non-mammalian cell line genomes. We previously showed that pooled, genome-wide CRISPR screens can be conducted in *Drosophila* S2R+ cells using plasmid transfection in combination with site-specific integration of an sgRNA-expressing cassette. Here, we report application of this approach to the *Anopheles* mosquito cell line Sua-5B. First, using optimized Polymerase III promoters to drive sgRNA expression, we conducted pilot screens of 7 genes that, as expected, rediscovered known genetic interactions with the drugs rapamycin or trametinib and the molting hormone ecdysone. Next, we scaled the approach to include all predicted *Anopheles* open reading frames based on the *Anopheles gambiae* PEST reference genome. In designing the library, we also optimized sgRNA designs for the Sua-5B cell line genome sequence and prioritized designs based on a machine-learning algorithm developed based on *Drosophila* CRISPR screen datasets. We then delivered the genome-wide library into Sua-5B cells at scale and determined which sgRNAs dropped out of the population over time, leading to identification of approximately 1,300 predicted fitness genes. As expected, these genes include most of the known or predicted components of essential complexes such as the ribosome, proteasome, and spliceosome. Lastly, we conducted proof-of-principle genome-wide positive selection screens to discover genes necessary for intoxication or infection of insects by pathogens. In one such screen, we identified a receptor and components of a putative internalization mechanism for an insect-crustacean toxin, and in another, we identified genes important for infection by an arthropod-born virus. Application of our approach has made genome-wide CRISPR knockout screens possible in cultured *Anopheles* mosquito cells for the first time, and provide a straightforward approach for conducting forward genetic screens in any organism for which derived cell lines are available.

620S **Gene-language are whole-genome representation learners** Charles Chen Biochemistry and Molecular Biology, Oklahoma State University

The language of genetic code embodies a complex grammar and rich syntax of interacting molecular elements. Recent advances in self-supervision and feature learning suggest that statistical learning techniques can identify high-quality quantitative representations from inherent semantic structure. We present a gene-based language model that generates whole-genome vector representations from a population of 16 disease-causing bacterial species by leveraging natural contrastive characteristics between individuals. To achieve this, we developed a set-based learning objective, AB learning, that compares the annotated gene content of two population subsets for use in optimization. Using this foundational objective, we trained a Transformer model to backpropagate information into dense genome vector representations. The resulting bacterial representations, or embeddings, captured important population structure characteristics, like delineations across serotypes and host specificity preferences. Their vector quantities encoded the relevant functional information necessary to achieve state-of-the-art genomic supervised prediction accuracy in 11 out of 12 antibiotic resistance phenotypes.

621S **Identification of Phytochemicals from Medicinal Plants as Strong Binders to SARS-CoV-2 Proteases ($3CL^{Pro}$ and PL^{Pro})**

and RNA-dependent RNA polymerase (RdRp) by Molecular Docking and Dynamic Simulation Studies Quaiser Saquib[1], Ahmed H Bakheit[2], Sarfaraz Ahmed[3], Sabiha M Ansari[4], Abdullah M AlSalem[5], Abdulaziz A AlKhedhairy[5] [1]Zoology Department, College of Science, King Saud University, [2]Pharmaceutical Chemistry, College of Pharmacy, King Saud University, [3]Pharmacognosy, College of Pharmacy, King Saud University, [4]Botany and Microbiology, King Saud University, [5]Zoology Department, College of Sciences, King Saud University

COVID-19, a disease caused by SARS-CoV-2, has caused a huge loss of human life, and the number of deaths is still continuing. Despite the presence of a few repurposed drugs and vaccines, the search for potential small molecules to inhibit SARS-CoV-2 is in demand. We provide promising computational (*in silico*) data on phytochemicals (compounds **1**-**10**) from Saudi medicinal plants as strong binders, targeting 3-chymotrypsin-like protease ($3CL^{Pro}$) and papain-like proteases (PL^{Pro}) and RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2. Compounds **1**-**10** followed the Lipinski rules of five (RO5) and ADMET analysis, exhibiting drug-like characters. Non-covalent (reversible) docking of compounds **1**-**10** demonstrated their binding in the active pocket with the catalytic dyad (CYS145 and HIS41) of $3CL^{Pro}$, catalytic triad (CYS111, HIS272, and ASP286) of PL^{Pro}, template primer nucleotides (A and U) and key amino acid residues (ASP623, LYS545, ARG555, ASN691, SER682, and ARG553) of RdRp. Moreover, the implementation of the covalent (irreversible) docking protocol revealed that only compounds **7**, **8**, and **9** possess the covalent warheads, which allowed the formation of the covalent bond with the catalytic dyad (CYS145) in $3CL^{Pro}$, catalytic triad (CYS111) in PL^{Pro}, and a crucial amino acid (CYS813) in the palm domain of RdRP. Root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg) analysis from molecular dynamic (MD) simulations revealed that complexation between ligands (compounds **7**, **8**, and **9**) $3CL^{Pro}$, PL^{Pro} and RdRP was stable, and there was less deviation of ligands. Overall, the *in silico* data on the inherent properties of the above phytochemicals unravels the fact that they may act as inhibitors for $3CL^{Pro}$ and PL^{Pro}. Also, the studied compounds exhibit non-nucleoside and irreversible interaction capabilities to inhibit RdRp that shed new scaffolds as antivirals against SARS-CoV-2. Nonetheless, to confirm the theoretical findings here, the effectiveness of the above compounds as inhibitors of $3CL^{Pro}$, PL^{Pro}, and RdRp warrants future investigations using suitable *in vitro* and *in vivo* tests.

Keywords: SARS-CoV-2; COVID-19; medicinal plants; $3CL^{Pro}$; PL^{Pro}; RdRp; docking; MD simulation; phytochemicals.

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622S **Development of a Computational Pipeline for Identifying Haplotype-Aware Genomic Variation in *Saccharomyces cerevisiae*** Joy A Love, Joseph A Stewart, Mackenzie A Wienke, Juan Lucas Argueso Environmental and Radiological Sciences, Colorado State University

Loss of Heterozygosity (LOH) is a class of chromosomal structural mutation that has important implications in the development of numerous genetic diseases, including cancer. LOH tracts occur when heterozygous genomic regions become homozygous for either one of the two available alleles. While using *Saccharomyces cerevisiae* as a model organism, we have identified LOH tracts in a mutation accumulation experiment. Accurate and efficient identification of LOH events from short-read whole genome sequencing (WGS) data is crucial for understanding these mutations. Currently, our group uses Nexus Copy Number to detect LOH tracts. However, this program has limitations: (1) it lacks haplotype phasing awareness, making it challenging to determine which allele a LOH clone tract became homozygous for, and (2) its commercial license is costly. The goal of this project is to develop a haplotype-aware and cost-effective LOH calling solution, leveraging Python and CLC Genomics Workbench. We conducted experiments to obtain LOH tracts in a hybrid heterozygous diploid strain background. We derived multiple yeast clones carrying genome-wide LOH tracts from this hybrid parent, and characterized them using the standard analysis above. These known LOH clones serve as the validation set for the new pipeline we are developing. To perform our new method, we first created a complete list of heterozygous single nucleotide polymorphisms (HetSNPs) in the parent strain. We validated this list by mapping reads from the fully heterozygous parent diploid to one of the haploid parents (our reference genome). We then derived a list of variants shared by the two sets and removed variants present at repetitive regions of the genome to arrive at a high-confidence HetSNP list. This enabled us to generate a custom variant tract in CLC. Subsequently, we mapped WGS reads from the known LOH clones above to the reference, creating a variant list for each LOH clone. These mapping variants were interrogated against the high-confidence HetSNP variant tract. CLC provided allelic identity and frequency outputs at all HetSNP positions, which were used in a custom Python code to make haplotype-aware phased genotyping calls. These calls were validated against known LOH tracts initially identified in Nexus. Our initial results show that this approach can reveal the full complexity of LOH tracts, including discontinuities in gene conversion segments near the recombination endpoints. As our study progresses, we will incorporate further insights and data into our presentation, providing a comprehensive overview and outlook of our research findings.

623S **Finding a function for Aim33 in *Saccharomyces cerevisiae*** Julia Iacovella, Thientrinh V Nguyen, Deborah Duong,

Analise Sulentic, John Giovinco, Julia Y Lee-Soety Biology, Saint Joseph›s University

Yeast *AIM33* encodes a protein with unknown molecular function, biological process, or cellular location. It is, however, 27% similar to human cytochrome b5 reductase CYB5R4, known for elongation of fatty acids, protection against oxidative stress, and cholesterol biosynthesis. *PGA3*, *AIM33*'s paralog, shares 55% similarity, and also encodes a cytochrome b5 reductase. Pga3 is able to extend chronological lifespan (CLS) under caloric restriction (CR) conditions, which limits typical caloric intake in nutrient-exhausted media without malnutrition. We compared the responses to CR between *aim33Δ* mutants and WT cells and observed that both cell types extended CLS in low sugar conditions indicating that Aim33 is not involved in caloric restriction responses. Interestingly, the *aim33Δ* mutants grew better than WT cells in minimal medium (SD) but grew similarly well in rich YPAD and synthetic complete (SC) media. Since Aim33 is likely a cytochrome b5 reductase with NAD and FAD binding domains, we measured and compared levels of NADH in WT and *aim33Δ* mutants and found that NADH levels were significantly lower in the mutants. Because *aim33Δ* mutants showed mitochondrial inheritance abnormalities, we stained for mitochondria in WT and mutant cells; no differences were observed. We did find that the *aim33Δ* mutation led to cells lingering longer in the S phase of the cell cycle and had decreased sporulation efficiency compared to cells with intact *AIM33*. In further exploration of *AIM33*, we discovered that it has genetic interactions with several genes with roles in telomere maintenance and in RNA processing. We generated *aim33Δ* haploid cells including deletions in *TLC1* which encodes the RNA template of telomerase and/or *NPL3* which encodes for an RNA processing protein involved in telomere maintenance. We measured and compared the rate of senescence which occurs when progressively shortened telomeres signal DNA damage and cause cell cycle arrest. While *tlc1Δ npl3Δ* double mutants senesced faster than *tlc1Δ* cells, the rates of senescence were similar between the *tlc1Δ* and *tlc1Δ aim33Δ* mutants and between *tlc1Δ npl3Δ* double and *tlc1Δ npl3Δ aim33Δ* triple mutants. Therefore, Aim33 likely does not have a role in supporting telomere maintenance. Furthermore, while genome-wide studies showed *aim33Δ* restoring the growth of *npl3Δ* cells at low temperature, we did not observe any differences in the rates of population doubling in *npl3Δ aim33Δ* mutants in standard growing conditions.

624S **Development of a yeast platform for expression and optimization of PET plastic degrading enzymes** Raphael Loll-Krippleber, Grant W Brown Department of Biochemistry and Donnelly Centre, University of Toronto

Over the past 70 years since the introduction of plastic into everyday items, plastic waste has become an increasing problem. With over 400 million tonnes of plastics produced every year, solutions for plastic recycling and plastic waste reduction are sorely needed. Recently, multiple enzymes capable of degrading PET (PolyEthylene Teraphthalate) plastic have been identified and engineered. In particular, the enzymes PETase and MHETase from *Ideonella sakaiensis*, have been shown to allow depolymerization of PET into the two precursors used for its synthesis, ethylene glycol (EG) and terephthalic acid (TPA). We have recently established a platform using *Saccharomyces cerevisiae* to develop a whole-cell catalyst expressing the MHETase enzyme, which converts MHET (monohydroxyethyl terephthalate) into TPA and EG, on the cell surface (PMID: 36587193). Following a similar strategy, we have now generated and assessed multiple construct architectures for efficient expression of PETase which acts upstream of MHETase and convert PET into MHET. Using a newly developed high-throughput plate assay that allows quantitative measurement of enzymatic activity, we demonstrate that despite differences between construct architectures, all PETase constructs are active against a model substrate. Importantly, expression of a published improved variant of PETase (FAST-PETase) increased activity of all PETase constructs, demonstrating that our plate assay and expression system recapitulates enzyme improvements characterized with purified enzymes. Using Synthetic Genetic Array (SGA), we introduced ~ 4200 non-essential gene deletions in our two best performing FAST-PETase expressing strains and measured PETase activity in the resulting strains using our high-throughput plate assay. We identified 54 gene deletions resulting in a robust increase in FAST-PETase activity, demonstrating the usefulness of our screening pipeline for PET-degrading strain improvement. Our current work aims to understand the mechanism by which gains in activity are achieved in these strains as well as screening gain-of-function and thermosensitive yeast essential genes libraries to identify further genetic alteration leading to PETase activity increases.

625S **Accelerated assembly of synthetic yeast chromosomes *in vivo* using DNA fragments with extensive synonymous recoding** Cara B Hull, Elizabeth Moore, Daniel Lusk, Ian Ehrenreich Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California

Homologous recombination in the budding yeast *Saccharomyces cerevisiae* is often used to assemble megabase-scale DNA molecules from smaller fragments. While this process works extremely well with DNA that has low identity to the *S. cerevisiae* genome, assembly of synthetic *S. cerevisiae* DNA *in vivo* poses unique challenges due to homologous sequences in the yeast genome. We hypothesized that a DNA synthesis strategy involving extensive synonymous recoding of protein-coding genes and use of recoded genes as assembly junctions could overcome this constraint. To test this hypothesis, we designed *in silico* a segment of *S. cerevisiae* Chromosome I that had identical intergenic regions and protein sequences to the natural chromosome but also possessed coding regions that differed at ~37% of nucleotides relative to natural homologs. We

then divided this design into 21 partially overlapping DNA fragments that were amenable to synthesis, with overlaps between fragments entirely restricted to coding regions. These DNA fragments were synthesized and co-transformed into yeast cells to assemble them with each other and a BAC/YAC vector. Here, we report the properties of this complex DNA assembly reaction and discuss our larger objective to use synonymous recoding schemes to build completely synthetic *S. cerevisiae* chromosomes in single assembly reactions involving large numbers of DNA fragments.

626S **What You Probably Didn't Learn When You Began Your Research Career – The Logic and Other Thoughts About How To Do Successful Research - Cliffnotes for Graduate Students and Post-Docs** Terrance G. Cooper Microbiology, Immunology & Biochemistry, University of Tennessee Health Science Center

The motivation for this work emanated from conversations with many graduate students and post-doctoral fellows. A simple question. How did you learn to do research? The answers were remarkably uniform. "Here's the project, here's the background, here are the techniques, let's begin." My objective has been to improve on that beginning. There is a logic to doing successful research, which graduate students and indeed post-doctoral fellows and young independent investigators often learn only with experience sometimes at their cost. The purpose of this work is to share the product of that experience and advice, working in nitrogen catabolite repression, Gln3, Gat1, Gcn2, and TorC1 as they begin their training and careers. In short, "CliffsNotes™" on how to think about and conduct a successful research program along with comments that have been guideposts for me as I have pursued the wonderful and winding road of nitrogen regulation in *Saccharomyces cerevisiae*. Anyone can walk on water if someone shows you where the stones are. Herein are some of the stones. FEMS Yeast Research FEMS Yeast Res. 23:foac065. doi: 10.1093/femsyr/foac065. PMID: 36881669 https://doi.org/10.1093/femsyr/foac065Supported by the Harriet S. VanVleet Chair of Excellence and NIH GM35642.

627S **A zebrafish model for whole-organism quantitative phenotyping of pleiotropy** Alex Y Lin[1], Maksim A Yakovlev[1], Ke Liang[2], Caroline R Zaino[1], Daniel J Vanselow[1], Andrew L Sugarman[1], Patrick La Riviere[3], Yuxi Zheng[4], Justin D Silverman[2], John C Leichty[5], Sharon X Huang[2], Damian B van Rossum[1], Victor A Canfield[1], KhaiChung L Ang[1], Keith C Cheng[1] [1]Pathology, Pennsylvania State University College of Medicine, [2]Information Sciences and Technology, Pennsylvania State University, [3]Radiology, University of Chicago, [4]Mathematics, Pennsylvania State University, [5]Marketing, Pennsylvania State University

Whole-organism phenotyping is necessary for the detection of pleiotropy, a frequent outcome of single-gene mutation and single chemical exposure. We are working towards a computational mechanism for microanatomic "histopathological" phenotyping of whole organisms, which would lead to a better understanding of the mechanisms underlying pleiotropy, gene function, chemical toxicity, and environmental influences such as nutrition, temperature, and disease. Since cells and tissues are 3-dimensional, a 3D histology has been needed to make such computational phenotyping possible. As a model for the objective evaluation of pleiotropic phenotypes, we are characterizing the dynamic phenotypes across organ systems in a null zebrafish mutant, *huli hutu* (*hht*) of the B subunit of DNA polymerase α, *pola2.* Homozygotes for this mutation evolve a complex pleiotropy associated with DNA damage and S phase arrest across multiple organ systems over 5-7 days, including nuclear atypia, a common cellular feature in human cancers and pre-cancers, in gastrointestinal organs, nuclear fragmentation in the eye and brain. As a first step toward quantitative, computational tissue phenotyping that includes pleiotropic phenotypes, physical characteristics of wild-type and *hht* blood cells were examined by micro-CT customized for histopathology, or X-ray histotomography, which generates 3D images of whole larvae at cellular resolution. Computer-assisted quantification revealed that the distribution of blood cells in the fish is conserved between wild-type and *hht* mutants, but significant, quantifiable differences were detected in cell count and cell dimensions. The application of geometric modeling across cell types and across organisms and sample types may serve as a computational foundation for more open, informative, rapid, quantitative, and reproducible whole-organism phenotyping.

628S **Process for developing optimized siRNA conjugates for *in vivo* delivery.** Sean F McCauley[1], Hannah McCauley[1], Kathleen Tran[1], Alexey Wolfson[1], Per Lindell[2] [1]ADViRNA, [2]Panther Therapeutics

siRNA conjugates are quickly becoming the leading approach in oligonucleotide drug development. This relatively new class of therapeutics informational drugs, consist of two different modalities; where functionality and gene specificity are provided by the sequence of the siRNA and *in vivo* targeting is achieved by the nature of the conjugate. As a result, delivery of siRNA conjugates to their target tissues *in vivo* does not require any additional formulation. To reach their targets *in vivo* and produce a long-term biological effect, the siRNA requires a heavily chemically modified backbone to confer nuclease resistance and functional longevity. However, these same chemical modifications significantly restrict the sequence space available for functional siRNAs. Consequently, their design presents a challenging task.

We present here a process for the efficient design, screening, characterization, and optimization of siRNA conjugates for

the discovery of maximally functional entities for research applications and therapeutic development. Using the example of VEGF-A siRNA design we will discuss potential pitfalls and complications associated with this process.

629S **The Monarch Initiative: An analytic platform integrating phenotypes, genes, and diseases across species** Monica C Munoz-Torres[1], Christopher J Mungall[2], Peter N Robinson[3], Melissa A Haendel[1] [1]Biomedical Informatics, University of Colorado Anschutz Medical Campus, [2]Biosystems Data Science, Lawrence Berkeley National Laboratory, [3]Berlin Institute of Health, Charité – Universitätsmedizin

Bridging the gap between genetic variations, environmental determinants, and phenotypic outcomes is critical for supporting clinical diagnosis and understanding mechanisms of diseases. It requires integrating open data at a global scale. The Monarch Initiative, an international consortium that aims to harmonize data across scientific disciplines to reveal disease mechanisms and aid disease diagnosis, advances these goals by developing open ontologies, semantic data models, and knowledge graphs for translational research. The Monarch App (monarchinitiative.org) is an integrated platform combining data about genes, phenotypes, and diseases across species. Monarch's APIs enable access to carefully curated datasets and advanced analysis tools that support the understanding and diagnosis of disease for diverse applications such as variant prioritization, deep phenotyping, and patient profile-matching. Monarch features a scalable, cloud-based infrastructure; simplified data ingestion and knowledge graph integration systems; enhanced data mapping and integration standards; and a recently refreshed user interface with novel search and graph navigation features. Furthermore, we have advanced Monarch's analytic tools by developing a customized plugin for OpenAI's ChatGPT to increase the reliability of its responses about phenotypic data, allowing us to interrogate the knowledge in the Monarch graph using state-of-the-art Large Language Models. The resources of the Monarch Initiative can be found at monarchinitiative.org and its corresponding code repository at github.com/monarch-initiative/monarch-app.

630S **Positional cloning of a chemically induced zebrafish mutant line with altered yolk lipid export** Nainika Pansari, McKenna Feltes, Steven A Farber Department of Biology, Johns Hopkins University

Lipids are essential nutrients for life and are needed for energy, act as chemical messengers, are signaling molecules and are integral components of cell membranes. Lipoproteins transport these hydrophobic molecules through the aqueous environments of metazoans (from insects to humans). In vertebrates, dietary lipids are absorbed/synthesized by the intestine and liver and are subsequently packaged into Apolipoprotein B-containing lipoproteins (B-lps). We still have an incomplete understanding of the genetic factors that influence B-lp biogenesis and metabolism. This is especially important since dysregulation of B-lp metabolism plays a major role in cardiovascular and metabolic diseases, that ultimately kill 25% of the human population. A large-scale forward genetic screen for a "dark yolk" phenotype associated with abnormal B-lp biosynthesis, in zebrafish, identified 27 mutant families. Here we report on the characterization and positional cloning of Mutant 5, which, in preliminary studies, was shown to have decreased B-lp levels. Using recombinant gene mapping and CRISPR/Cas9 editing, we identify the amino acid transporter ***slc3a2a*** gene as the molecular locus in the Mutant 5 line that produces the dark yolk phenotype. The identification of ***slc3a2a*****,** a gene previously unlinked to B-lp metabolism, that is conserved in humans**,** provides potentially new insight into the molecular players involved in regulation of vertebrate B-lps.

631V **REDfly: Uses of the Regulatory Element Database for *Drosophila* and other insects** Soile V. E. Keränen[1], Xinbo Huang[2], Marc S. Halfon[3,3,4] [1]None, [2]Center for Computational Research, University at Buffalo-State University of New York, [3]NY State Center of Excellence in Bioinformatics & Life Sciences, [4]Departments of Biochemistry, Biomedical Informatics, and Biological Sciences, University at Buffalo-State University of New York

REDfly is a knowledgebase of experimentally validated *cis*-regulatory modules (CRMs) and transcription factor binding sites (TFBSs) for *Drosophila melanogaster* and other insects, including the mosquitoes *Anopheles gambiae* and *Aedes aegypti* and the beetle *Tribolium castaneum*.

REDfly (v9.6.2 update) contains data on about 58,900 tested or predicted regulatory sequences and about 2700 TFBSs or their variants, with more being curated. We also have implemented JBrowse, which allows inspection of REDfly *D. melanogaster* data against the FlyBase reference genome, and UCSC PhastCons and PhyloP. The uses of REDfly vary from detailing the regulatory structure of a single locus, to large-scale studies of the regulatory genome, to providing training and/or validation data for machine-learning analyses of gene regulation. In other words, the REDfly data can be used for studying gene regulation for its fundamental rules and for developing methodology, both of which are applicable beyond *Drosophila* to metazoans in general.

CRM data in REDfly are broadly segregated into three categories: reporter gene analysis (CRM, RC), deletion of potential regulatory sequences from the genome (CRM segment), and predictions based on, e.g., ChIP-seq, ATAC-seq and/or computational modeling (pCRM). Also, we use the CRM data internally to infer minimally active sequences from tested CRMs

(iCRM). Transcription factor binding sites are mapped to overlapping CRMs.

CRMs or gene loci can be searched at http://redfly.ccr.buffalo.edu/search.php by entering a gene or CRM name. As REDfly CRM activity is annotated using structured ontologies, it is possible to search by using anatomy or GO terms to find CRMs active in specific organs or under certain conditions.

Search results can be downloaded in various formats or opened in individual palettes, which contain data and metadata for the regulatory element including sequence, activity profile, links to the source papers of the data, and links to our dedicated JBrowse genome browser, allowing the user to further explore the data. REDfly also curates silencers, which inhibit gene transcription, often while acting as enhancers for some other location or condition. Searching 'Including silencer' will return CRMs with silencing function, whereas 'Excluding enhancer' will return CRMs with only silencing function recorded.

REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed at @REDfly_database ("X") or at @REDfly@mstdn.science.

632V **split-intein Gal4: a potent intersectional genetic labeling system which can be controlled temporally via Gal80** Benjamin Ewen-Campen[1], Haojiang Luan[2], Jun Xu[3], Rohit Singh[4], Neha Joshi[1], Tanuj Thakkar[1], Bonnie Berger[5], Benjamin White[2], Norbert Perrimon[1] [1]Harvard Medical School, [2]National Institute of Mental Health, NIH, [3]CAS Key Laboratory of Insect Developmental and Evolutionary Biology, [4]Duke, [5]Massachusetts Institute of Technology

The existing split-Gal4 system allows for intersectional genetic labeling of specific cell types with exquisite spatial control. However, unlike native Gal4, it cannot be controlled temporarily because it cannot be repressed by Gal80. We have developed a split-Gal4 system based on a self-excising split-intein, which drives transgene expression as strongly as the current split-Gal4 system and Gal4 reagents, yet which is repressible by Gal80. We call this system "split-intein Gal4" and demonstrate its broad applicability in vivo. Further, we show that our split-intein Gal4 can be extended to the drug-inducible GeneSwitch system, providing an independent method for intersectional labeling with inducible control. We demonstrate that the split-intein Gal4 system can be used to generate highly cell type–specific genetic drivers based on in silico predictions generated by single-cell RNAseq (scRNAseq) datasets, and we describe an algorithm ("Two Against Background" or TAB) to predict cluster-specific gene pairs across multiple tissue-specific scRNA datasets. We have created a publicly available plasmid toolkit to efficiently create split-intein Gal4 drivers based on either CRISPR knock-ins to target genes or using enhancer fragments. Altogether, the split-intein Gal4 system allows for highly specific intersectional genetic drivers that are inducible/repressible.

633V **A Simple Assay for the Detection of Late-Stage Apoptosis Features in *Saccharomyces cerevisiae*** Narendra K Bairwa[1,2], Heena Shoket[3], Monika Pandita[3], Meenu Sharma[3] [1]Shri Mata Vaishno Devi University, [2]Central University of Jammu, [3]Genome Stability Regulation Lab, School of Biotechnology, Shri Mata Vaishno Devi University

Unicellular eukaryotic organisms such as yeast and protozoa serve as useful models for studying the impact of chemicals on cell physiology, cellular growth, and genome duplication. The yeast Saccharomyces cerevisiae has been widely used to assess apoptosis induced by chemicals due to its genetic tractability, ease of evaluation, and readily available impact assessment tools. Apoptosis in S. cerevisiae is characterized by many features, including increased cell death, loss of membrane integrity, release of caspases, chromatin condensation, and nuclear fragmentation, which are similar to the ones observed in mammalian cells. Current methods of apoptosis assessment typically require specialized equipment and reagents, which limits wide adoption. Here, we describe a rapid, inexpensive, and easy-to-perform assay in yeast for the analysis of late-stage apoptotic features in cells treated with a chemical. We describe a protocol for assessing loss of cell survival and changes in the nucleus. We demonstrate the approach by using acetic acid and hydrogen peroxide as test chemicals. This assay for the study of late-stage apoptotic features in *S. cerevisiae* can be performed reliably and rapidly by any laboratory with basic equipment and may be extended for studying apoptosis in similar single-cell organisms after treatment with toxicological agents

Keywords: *Saccharomyces cerevisiae,* DAPI staining; acetic acid; apoptosis; fluorescence microscopy; hydrogen peroxide

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634V **AVITI nucleic acid sequencing and its application** Jianhua Luo[1], Silvia Liu[2], Yanping Yu[1] [1]Pathology, University of Pittsburgh, [2]University of Pittsburgh

DNA sequencing is a critical tool in modern biology. Over the last two decades, it has been revolutionized by the advent of massively parallel sequencing, leading to significant advances in the genome and transcriptome sequencing of various

organisms. Nevertheless, challenges with accuracy, lack of competitive options and prohibitive costs associated with high throughput parallel short-read sequencing persist. Here, we conduct a comparative analysis using matched DNA and RNA short-reads assays between Element Biosciences' AVITI chemistry and Illumina's NextSeq 550. Similar comparisons were evaluated for synthetic long-read sequencing for RNA and targeted single-cell transcripts between the AVITI and Illumina's NovaSeq 6000. For both DNA and RNA short-read applications, the study found that the AVITI produced significantly higher per sequence quality scores. For PCR-free DNA libraries, we observed up to a 10-fold lower experimentally determined error rate for using the AVITI chemistry compared to the NextSeq 550. For short-read RNA quantification, both AVITI and the NextSeq 550 demonstrated comparable accuracy. With regards to synthetic long-read mRNA and targeted synthetic long read single cell mRNA sequencing, both platforms' respective chemistries performed comparably in quantification of genes and isoforms. The AVITI displayed a marginally lower error rate for long reads, with fewer chemistry-specific errors and a higher mutation detection rate. These results point to the potential of the AVITI platform as a competitive candidate in high-throughput short read sequencing analyses when juxtaposed with the Illumina NextSeq 550.

635V **Rareinsight: a collaborative rare disease report generator empowering clinicians and patients** Kimberly C Coetzer[1], Olaitan Awe[2,3] [1]Biomedical Sciences, Stellenbosch University, [2]African Society of Bioinformatics and Computational Biology, [3]University of Ibadan

The scarcity of knowledge surrounding rare diseases underscores the critical necessity for collaborative research efforts to unravel their complexities and improve diagnostic and therapeutic outcomes. This research introduces RareInsight, an effort focused on developing an open-source, interactive dashboard tailored for clinicians and patients. Its primary objective is to transform the interpretation of genetic variant data into flexible and detailed reports, aiming to ease rare disease diagnosis and research.

RareInsight will be designed to process genetic variant data into customizable, interactive reports. These files are preferably generated by nf-core's raredisease pipeline as it employs some level of filtering and prioritization from whole genome or whole exome sequencing data. The resulting VCF files will be used as input into the dashboard which is equipped with advanced filtering options, statistical analysis capabilities, and diverse export formats, empowering users to explore detailed data and seamlessly collaborate with peers for further analysis and interpretation. The final interactive dashboard will be developed using Shiny and tested using rare disease data from esteemed sources such as the Undiagnosed Disease Program and NHGRI GREGOR Consortium datasets found in dbGaP.

By encapsulating clinical relevance, genetic implications, ACMG classification, and essential resources within these reports, RareInsight will aid in informed decision-making for both clinicians and patients. Employing visual aids such as charts and graphs will further enhance comprehension and clarity. RareInsight will eliminate the proprietary limitations often present in existing tools. By adopting an open-source environment, it strives to encourage transparency and customization tailored to specific clinical or research requirements. The dashboard will also prioritize stringent data privacy and security measures to ensure the protection of sensitive genetic data.

Overall, this dashboard thrives on collaboration, by allowing researchers and clinicians to seamlessly share and collaborate on reports, fostering knowledge exchange and enriching the collective understanding of rare diseases. In essence, RareInsight is poised to redefine rare disease diagnosis and research by inviting collaboration and innovation to enhance healthcare outcomes in an open-source format.

636V **Resource-explicit interactions in spatial population models** Samuel Champer[1], Bryan Chae[1], Benjamin Haller[1], Jackson Champer[2], Philipp Messer[1] [1]Computational Biology, Cornell University, [2]Center for Bioinformatics, Peking University

Continuous-space population models can yield significantly different results from their panmictic counterparts when assessing evolutionary, ecological, or population-genetic processes . However, the computational burden of simulating spatial models is typically much greater than that of panmictic models due to the overhead of determining which individuals interact with one another and how strongly they interact. Though these calculations are necessary to model local competition that regulates the population density, they can lead to prohibitively long runtimes. Here, we present a novel modeling method in which the resources available to a population are abstractly represented as an additional layer of the simulation. Instead of interacting directly with one another, individuals interact indirectly via this resource layer. We find that this method closely matches other spatial models , yet can increase the speed of the model by an order of magnitude, allowing the simulation of much larger populations. Additionally, models structured in this manner exhibit other desirable characteristics, including more realistic spatial dynamics near the edge of the simulated area, and an efficient route for implementing more complex heterogeneous landscapes.

637T **Metabolic-based Polyclonality in Genetically-induced Intestinal Tumors** Pierre S Delamotte CNRS - I2BC

Cancers are usually considered of monoclonal origin, i.e arising from a unique cell, and their energy metabolism considered to rely on glucose fermentation to lactate and not on mitochondrial respiration. Either characteristics have been challenged by a number of studies. The goal of the present study is to decipher tumor metabolism and to demonstrate polyclonality using a Drosophila midgut tumor model. These tumors are induced at a chosen time and express GFP. They result from the loss of the tumor suppressor Apc (Adenomatous polyposis coli) and express an oncogenic form of Ras(RasV12). These two genetic events are typically found in human colorectal cancers. Here, we generated fly lines to evaluate the role of metabolic enzymes in those tumors and to follow their polyclonality based on a metabolic heterogeneity. We showed by cytometry that glycolysis, lactic fermentation, mitochondrial respiration, fatty acid synthesis, neoglucogenesis and glutamin-dependant anapleurosis are necessary to tumoral growth. We observed in microscopy that tumors are always formed by several clones and display a metabolic diversity. Furthermore, single cell RNAseq reveals several clusters of metabolically distinct tumor cell subpopulations. Finally, recent live imaging, both using flybow and biosensors suggests a high motility of the already metabolically-specialized tumor clones. The hypothesis, based on these results, is that several clones from different midgut locations have to associate and cooperate to ensure complementary metabolic functions. Demonstrating mandatory polyclonality in tumors, that are systematically generated with the same genetic modifications, will bring a novel vision to adapt to each cancer type the appropriate therapeutic treatment.

638T **Characterization of bacterial dynamics within the *Caenorhabditis elegans* intestine** Andrew N Moore, Justin Ellis, Erin Nishimura, Jessica Hill Biochemistry and Molecular Biology, Colorado State University

The gut microbiome is a key regulator of health, modulating various aspects of host physiology such as immunity and metabolism. However, our understanding of fundamental processes underlying interactions between the gut microbial community and host cells remains incomplete. *Caenorhabditis elegans* is an attractive model organism for studying host-microbe interactions in the gut, featuring stereotyped development of a 20-cell intestine which lacks an adaptive immune system. Previous work has profiled the natural microbiome of the nematode using 16S rRNA analysis, and a core microbial community of 12 bacterial strains known as CeMbio is available for studies of the *C. elegans* microbiome. Here, we characterize the spatial dynamics of bacterial colonization in the intestinal lumen of JUb66 (*L. amnigena*, a CeMbio community member) as both a monoculture and as a part of the CeMbio community. We transformed the CeMbio community members using a mini-Tn7 insertion system to create RFP reporter bacteria. We then fed *C. elegans* these bacteria to image using fluorescence microscopy and processed the images using tools from WormBiome Viewer to determine bacterial spatial patterning in the intestinal lumen. We found that JUb66 preferentially colonizes the mid to posterior region of the intestinal lumen as a monoculture, while its localization shifts primarily to the middle with reduced abundance as part of the community. This suggests that coordination of the microbiome and colonization by specific bacteria or a simplified bacterial community may not be finely localized to select intestinal cells throughout the intestine, but rather to larger regions of the intestine. In comparison, *E. coli* (OP50), the standard lab diet, showed similar spatial preference throughout the intestinal lumen but displayed decreased abundance when compared with JUb66, suggesting a reduced ability to colonize the host. Overall, these data provide a glimpse into whether bacteria maintained within the *C. elegans* intestine display spatial preference. Together, this system allows for future experiments which modulate the host transcriptome to observe effects in the microbiome and is a first step in understanding the underlying mechanisms of microbiome selection and maintenance.

639T **WormCat 2.0 defines characteristics and conservation of poorly annotated genes in *Caenorhabditis elegans*** Amy K Walker[1], Daniel Higgins[2] [1]Program in Molecular Medicine, UMASS Medical School, [2]PMM, UMASS Medical School

In today's post-genomic era, we know that most metazoans genomes encode around 20K genes. It is striking that nearly half of these genes are sparsely annotated, and many others are described by domain conservation. This profoundly biases gene enrichment studies, as the most well studied pathways have the most annotations per gene and limits studies into less well understood systems. For example, of 621 C. elegans transcription factors, the top 20 account for 40% of papers and have more than 2X the number of GO terms per gene.

We developed WormCat, a gene categorization tool for identifying and visualizing enrichment in genome-scale data, which depended on a custom annotation of the C. elegans genome. The WormCat annotation strategy differed from GO in two major ways. First, each gene received a single nested annotation with broad to more specific functions. These categories were based on physiological function, molecular or location-based category. Second, genes with little associated information were placed in a specialized category, UNASSIGNED. This allows poorly annotated genes (PAGS: UNASSIGNED and domain-based categories, such as TRANSMEMBRANE DOMAIN) to be tracked in transcriptomic experiments. We noted that nearly half the genes expressed in neurons or intestine from published studies were poorly annotated. One hypothesis is lineage specificity; however, we find that PAGs may have orthologs outside of C. elegans, which also lacked functional characterization. We find several indications that some of these genes may be amenable to functional characterization, such as phenotypes in large scale RNAi screens, which suggest functional testing has not been saturated. Using WormCat to interrogate a wide variety

of published RNA seq data, we also find that UNASSIGNED genes may be enriched in specific tissues or stress conditions, suggesting response to specific regulatory cures. In order to classify these understudied genes, we are using machine learning tools to cluster PAGs with genes of known function for detailed phenotyping studies. Conserved but functionally uncharacterized genes may function outside the lab environment, contain redundant functions, or support robustness. Many -omics studies depend on pathway analysis or previous annotation to select genes for functional analysis. Developing tools to improve functional gene annotation is critical for evaluating -omics data and identification of the most biologically relevant candidates for future study.

640T **A comparative study of piRNA evolution in *Drosophila simulans*.** Prakash Narayanan[1], Sarah Signor[2] [1]North Dakota State University, [2]Biological Science, North Dakota State University

Transposable elements (TE) are selfish genetic elements, capable of replicating themselves in a host genome. In germ cells TE replication is repressed by a special class of non-coding small RNA called piRNA, which originate from discrete loci in the genome called piRNA clusters (piCs). These loci harbor remnants of TEs that play an important role in defense against TE invasion. While there are many studies that have clarified the transcription and specification of piRNA clusters, little is known about the evolution of piRNA clusters. The two popularly proposed models of piRNA cluster evolution are the 'de novo' and 'trap' models. The 'de novo' model suggests that a newly inserted active TE can be transformed into a piRNA cluster by the production of small amounts of cognate piRNA. This new TE insertion turned piRNA cluster will prevent future invasions of the particular sequence of TE. The 'trap' model proposes that large evolutionarily conserved piRNA clusters act as traps for TEs, thus a TE invasion is stopped when one invading TE copy jumps into the piRNA cluster at random , leading to the cluster producing piRNAs. This ability of the cluster remains for long-term and hence piCs can produce more piRNAs that stop future invasion of related TEs . Recent evidence suggests that piRNA clusters evolve along a continuum of these models with aspects of each in *Drosophila melanogaster*. However, in *Drosophila simulans* , the model organism we are interested in studying, we see different patterns in piRNA cluster evolution compared to *D. melanogaster*. For example, only few classes of TEs seem to nucleate the formation of piRNA clusters in *Drosophila melanogaster. In contrast*, a large variety of classes of TEs seem to nucleate piRNA clusters. PiRNA production is more diffuse than in *D. melanogaster*, and we see more aspects of the *de novo* model in general. A common factor shared between the species seems to be that many piRNA clusters are genotype-specific. Overall our study looks to bring an evolutionary perspective to piRNA clusters, this will help us to understand what is necessary for TE suppression, what is expendable, and how TE suppression system evolve in response to new invasions.

641T ***m6A* patterns are consistent across different *Drosophila* datasets and regulates alternate splicing** George Boateng-Sarfo[1], Lijuan Kan[2], Sarah Signor[3], Eric Lai[2,2] [1]Genomics, Phenomics and Bioinformatics, North Dakota States University, [2]Developmental Biology Program, Memorial Sloan Kattering Cancer Center, [3]Biological Science, North Dakota States University

Methylation of adenosine at the *N*-6 position (m^6A) is the most common internal RNA modification in eukaryotes. It has been hypothesized to play significant biological roles including alternate splicing, RNA decay, neural function, and sex determination. However, there are many artifacts in m^6A data that potentially preclude these conclusions. Here, we are developing an atlas of m^6A from publicly available data. We mapped m^6A genomic regions in wild-type and knocked-out samples of Whole fly, Head, Neuron, Neuroblast, Schneider cells (S2 cells), and Embryo samples. Each dataset is assessed for quality and excluded from the meta-analysis if the data collection was flawed. The included datasets are cross-referenced to identify artifacts affecting m6a calling. Using this approach we have identified a number of different patterns in m^6A data that alter some of the existing conclusions about RNA modifications. First, we have found that in Drosophila m^6A modifications are primarily enriched in the 5' Untranslated Regions (5' UTR). Second, we showed that m^6A modification patterns do not vary significantly across samples or tissue although biological roles and mechanisms vary. We finally provide an atlas that represents the distribution of m^6A across the epitranscriptome of varying tissues. In conclusion, we show that m6A is significantly enriched in 5'UTR of flies compared to mammalian cells although the methylome pattern is consistent across different datasets.

642T **The *Drosophila melanogaster* microbiome is modified by parasitic nematode infection** Raymond Yau[1], Ioannis Eleftherianos[2] [1]Biological Sciences, George Washington University, [2]George Washington University

The gut microbiome is found in the gastrointestinal system and provides its host with health advantages, particularly by controlling immunological homeostasis. The fruit fly *Drosophila melanogaster* is a vital model for studying the microbiome due to the availability of genetic resources and procedures. To understand the importance of microbial composition in shaping immune modulation, it is imperative to investigate the functional role of the microbiota through parasitic infection. To achieve this, we use entomopathogenic nematodes (EPN) of the genus *Steinernema* which exhibit remarkable ability to swiftly and efficiently infect a diverse array of insect species, facilitated by the mutualistic bacteria found within their gut. *Steinernema carpocapsae* forms an obligate mutualistic association with the Gram-negative bacteria *Xenorhabdus nematophila*, which is an excellent model to study pathogen infection processes and host anti-nematode and antibacterial immune

responses. *Steinernema hermaphroditum* harbors the mutualistic bacteria *Xenorhabdus griffiniae* and this nematode produces hermaphrodites in the first generation and males and females in the second generation. This study aims to examine the microbiome changes in *D. melanogaster* larvae in response to *S. carpocapsae* and *S. hermaphroditum* nematode infection. For this, *D. melanogaster* late second to early third instar Oregon-R larvae were exposed separately to *S. carpocapsae* TT01 and *S. hermaphroditum* CS34 in 96 well plates using our standard EPN infection assay. We have found that *S. carpocapsae* infective juveniles are more pathogenic to *D. melanogaster* larvae compared to the closely related *S. hermaphroditum*. Our preliminary analysis also indicates substantial changes in the size and composition of the *D. melanogaster* larval microbiome during infection with EPN nematodes compared to the uninfected controls. The obtained results serve as a foundation for succeeding studies to elucidate the EPN-specific effector molecules that alter the *D. melanogaster* microbiome and understand the role of the microbiome in regulating insect anti-nematode immune processes.

643T **R-loop formation at transposable and repetitive elements in *Drosophila melanogaster* ovaries** Timothy J Stanek, Adam Kneebone, Weihuan Cao, Christopher E Ellison Genetics, Rutgers University

R-loops are three-stranded nucleotide structures consisting of a DNA:RNA hybrid and a displaced ssDNA non-template strand. Originally viewed as byproducts of transcription, R-loops are now recognized as important regulators of gene expression and genomic stability. Persistent dysregulation of R-loop maintenance can result in replication stress, DNA double-strand breaks, and chromosomal rearrangements that contribute to diseases such as neurological disorders and cancer. However, the full scope of R-loop-mediated contributions to genomic instability have not yet been explored.

Aberrant activation of transposable elements (TEs) has been a well-documented source of genomic instability and disease, including their insertion into genes and their imposition of epigenetic effects on nearby loci. However, the extent to which their disruptive effects involve concomitant or subsequent formation of R-loops remains unknown.

Here we used DNA:RNA immunoprecipitation followed by high-throughput sequencing (DRIP-seq) to map the R-loop profiles of *D. melanogaster* at TEs and repetitive DNA elements. To ensure detection of *cis* R-loops at active TEs, we performed DRIP-seq in the germline of control and *rhino* knockout flies, whose loss of piwi-interacting RNA (piRNA) expression results in derepression of TEs. We observe that R-loops form primarily in LTR-type retrotransposons from the Gypsy family, which contain DNA sequence motifs favorable to R-loop formation. Moreover, derepression of TEs in $rhi^{-/-}$ ovaries correlates with R-loop formation, with *rhi*-induced R-loops forming primarily in the LTR region of specific LTR-type TEs. In the Drosophila Genetic Reference Panel (DGRP), polymorphic copies of these same R-loop-forming TEs show reduced frequencies compared to non-R-loop forming TEs, suggesting they are under increased purifying selection, potentially due to detrimental effects of R-loop formation on genome stability. We also detect significant R-loop formation at many satellite repeats, yet in contrast to TEs, *rhi*-induced changes in satellite expression appear to occur independently of R-loop formation. Collectively, these results reveal a specialized relationship between TE expression, TE activity, and R-loop formation.

644T **Transcriptome Analysis of the Effects of Loss of ft and Deletion of Conserved Domains in Imaginal Wing Disc** Nattapon Thanintorn[1], Cole Julick[1], Jannette Rusch[1], Megan Glaser[1], Yonit Tsatskis[2], Yi Qu[3], Hongtao Zhang[3], Hyunseo Oh[4], Helen McNeill[1] [1]Developmental Biology, Washington University School of Medicine, [2]Cell Biology, The Hospital for Sick Children, [3]Lunenfeld-Tanenbaum Research Institute, [4]University of Toronto

During animal development, Fat (Ft), an enormous cadherin with extracellular domains of 32 cadherin repeats, contributes to precisely controlled organization at the cellular and tissue level by regulating growth of a tissue and establishing orientation of cells within the tissue. Drosophila Fat and its human ortholog FAT4 share 6 highly conserved cytoplasmic regions. Loss of *ft* in flies leads to pupal lethality, massive overgrowth of imaginal discs, and loss of planar cell polarity in tissue. Mutations in FAT4 in human causes cancer and Van Maldergem and Hennekam syndromes. Extensive studies from our group and others revealed that *ft* can reduce tissue overgrowth via the Hippo signaling pathway. However, little is known about how the critical regions of *ft* contribute to mechanisms in growth control and cell polarity. We have used an extensive CRISPR-based mutagenesis of Drosophila Ft to dissect Ft function *in vivo*. Here we present transcriptome analysis of CRISPR-based mutagenesis of Ft in imaginal wing disc. Understanding how Fat regulates tissue growth and organization will generate new hypotheses to better understand how mutations of Fat cause human diseases.

645T **Roles of Transposable Elements in rewiring 3D genome architecture** Harsh Girish Shukla, Grace Yuh Chwen Lee Ecology and Evolutionary Biology, University of California, Irvine

The entirety of an organism's genetic material needs to fit into a very tight space in the cell nucleus. Understanding the underlying principles for the intricate 3D folding of genetic material is of utmost importance since it influences a wide variety of genome functions. This study aims to investigate the contribution of transposable elements (TEs) in shaping this 3D nuclear architecture. TEs are genomic parasites that promote their own spread at the expense of host fitness. In concert, the host

has evolved a variety of mechanisms to suppress this selfish behavior, in particular, the enrichment of suppressive repressive marks, such as H3K9me2/3. These marks enriched at TEs oftentimes spread into adjacent regions, including genes, turning the TE local regions into an island of heterochromatin within euchromatin. Previous studies have shown that H3K9me2/3-enriched regions, including TEs, in the euchromatin can spatially interact with pericentromeric heterochromatin (PCH) even though large linear distances might separate them. In Drosophila populations, most TE insertions are polymorphic, and many of them are found in few individuals. We thus hypothesize that this polymorphic nature of TEs can drive polymorphic 3D nuclear structure through their spatial interactions with PCH. To test our hypothesis, we generated Hi-C libraries for two inbred D. melanogaster strains. In order to mitigate mapping biases arising from using a single reference, we generated reference quality assemblies using highly accurate Pacbio HiFi for these two strains. The assemblies have a more complete representation of PCH regions than the current Drosophila reference. We developed a novel computational method to rescue some of the multi-mapped HiC reads originating from the highly repetitive PCH and derived normalized PCH-contact frequencies to compare homologous alleles with and without TE insertions between the two strains. Our analysis indeed identified TE-adjacent regions showing significantly more frequent interactions with PCH than the TE-free homologous allele. We are in the process of identifying biological attributes, such as type of TEs and H3K9me2/3 enrichment level, that make these regions more likely to be in 3D contact with PCH. Our investigation will reveal how TEs contribute to varying 3D genome organization, paving the way for further in-depth studies investigating their functional effects.

646T **Activity of a *Drosophila teissieri* I-element retrotransposon in *D. melanogaster*** Denise Clark, Lauren Miller Biology, Univ New Brunswick

I-elements are non-LTR (long terminal repeat) retrotransposons found in Drosophila species. Like human LINE1 elements, they have an internal promoter and two open reading frames encoding an RNA-binding protein (ORF1) and an endonuclease/ reverse transcriptase (ORF2). The binding of human LINE1 ORF1 protein (ORF1p) to RNAs other than its own can lead to gene duplications (retrocopies). Retrocopies can then evolve into either functional genes or pseudogenes. Although retrocopies have been detected in *D. melanogaster*, it is not known if I-element activity contributes to their production. Notably, the piRNA pathway typically represses I-element activity in *D. melanogaster*. However, crosses between females lacking I-elements (responder strain) and males with I-elements (inducer strain) can produce sterile F1 females that have elevated I -element transposition—a phenomenon known as I-R dysgenesis. Moreover, I-element de-repression can occur in Drosophila interspecific crosses. Earlier research showed that a *D. teissieri* I-element transformed into a *D. melanogaster* responder strain could transpose and induce I-R dysgenesis.

Our interest lies in further understanding I-element activity in the germline, particularly its potential role in retrocopy generation. To this end, we cloned a *D. teissieri* I-element (*Dt-I*), revealing a 99% similarity to an element isolated by others. We generated both epitope-tagged *HA-ORF1* and non-tagged transgenes to study expression, activity, and localization in the *D. melanogaster* female germline. We found the full-length untagged *Dt-I*, transformed into the responder strain w^K, is transcribed and capable of transposition, demonstrating its functional autonomy. The expression of HA-ORF1p in oogenesis, in the context of a full-length I-element, localizes to oocytes during stages 2-10 of egg chamber development, consistent with previous studies. *nanos-GAL4*-induced HA-ORF1p expression, without the context of the full-length I-element, is similarly localized. To determine if HA-ORF1p binds to its own RNA and other RNAs during oogenesis, as observed with LINE1 ORF1p in human cells, we conducted RNA immunoprecipitation (RIP) followed by next-generation sequencing. The RIP using an anti-HA antibody showed specific enrichment for *HA-ORF1* RNA compared to input RNA and non-specific antibody RIP. Collectively, these findings suggest that transport of *D. teissieri* ORF1p to the oocyte is specifically accompanied by its own RNA.

647T **High-throughput transposition analysis of hybrid dysgenesis in *Drosophila virilis* using Oxford nanopore sequencing** Ekta Mohanty, Chris Stillman, Justin Blumenstiel Ecology and Evolutionary Biology, University of Kansas

Transposable elements (TE) are genetic parasites that can cause DNA damage and genome instability by replicating themselves within the host genome. To study the genomic causes and impacts of transposition we use hybrid dysgenesis (HD) in *Drosophila virilis* as a model. HD is a phenomenon of sterility that occurs when a TE family present in the paternal genome, but absent in the maternal genome, becomes activated in the germline. If a female lacks a particular TE family in her genome, she is unable to provision corresponding piRNAs to her offspring. In the absence of these piRNAs, TEs from this paternally inherited family become mobilized in the germline, causing chromosome breaks and dysgenesis. Unlike other syndromes of hybrid dysgenesis, the *D. virilis* syndrome is associated with mobilization of multiple TE families. It is not clear if this joint mobilization is driven through the same mechanism (lack of maternal piRNAs) or whether the activation of some TEs triggers the activation of others. To characterize the global transposition profile during hybrid dysgenesis in *D. virilis*, we are employing Oxford nanopore sequencing. In our first pass, we used pooled sequencing of backcross progeny to identify new TE insertions. We are now developing an approach that uses single flies to characterize both insertions and excisions to compare transposition rates in the germlines of flies under dysgenic and non-dysgenic conditions. This transposition analysis will be

performed to further determine if piRNA asymmetry is globally predictive of transposition rates and what explains variation in the transposition rate across individuals.

648T **Live Imaging Analysis of Akirin/NuRD Complex Interactions During Cardiac Development** Ariana Craft[1], Belle Lea[2], Scott Nowak[2] [1]Cellular and Molecular Biology, Kennesaw State University, [2]Kennesaw State University

Congenital heart disease is one of the most widely reported human congenital malformations, yet the exact genetics behind this spectrum of maladies remains unknown. The severity of heart defects can range from minor to severe, with some of the most severe cases being deformed or missing heart valves. Previous studies have identified the conserved nuclear protein Akirin as an important yet relatively unstudied regulator of gene expression for cardiac development. Our data suggests Akirin works with the Nucleosome Remodeling and Deacetylase (NuRD/CHD) complex to effectively control the cardiac developmental program. We are currently conducting an analysis of genetic interactions between akirin and components of the NuRD/CHD complex. Our data indicates that akirin genetically works with several components of this multiprotein complex including MTA-like to properly regulate the cardiac gene expression program during development. Using a combination of fixed and live microscopic imaging techniques, we have determined that these interactions are critical for proper cardiac function in akirin mutants.

649T **Long-range spatial interactions of transposable elements impose trans epigenetic effects** Yi Gao, Grace YC Lee Ecology and Evolutionary Biology, University of California, Irvine

The spatial organization of the genome plays a critical role in gene regulation and genome stability. However, highly abundant selfish genetic elements, transposable elements (TEs), can challenge the integrity of such spatial organization. TEs copy and move themselves to other genomic positions, which could disrupt functional elements while contributing to genetic variation. Due to the disruptive nature of TEs, eukaryotic hosts evolve strategies to epigenetically silence euchromatic TEs by enriching them with repressive epigenetic marks, particularly H3K9me2/3. Recent studies across eukaryotes reveal that the repressive epigenetic marks can spread from euchromatic TEs into adjacent genes and perturb gene expression. In addition, H3K9me2/3 enriched euchromatic TEs were shown to be involved in long-range spatial interactions with the pericentromeric heterochromatin (PCH) domain through phase separation mechanisms. Building upon these observations, we hypothesize that TEs' spatial interactions with PCH domains will drive the silencing of both homologous alleles throughout the genome. This is because of the somatic pairing of the homologous chromosome, which can lead to both homologous alleles coming into contact with the PCH domain through TE-PCH spatial interactions. Thus, TEs could impose "trans-silencing" effects on both homologous alleles even when they are present in only one of the two homologous chromosomes. To test the presence of our proposed trans epigenetic effects, we crossed two DSPR founder lines that have distinct TE insertion profiles to generate F1 with heterozygous TE insertion sites. We performed CUTnTAG on the embryo to assay genome-wide H3K9me3 enrichment levels on the parent-offspring trio. We are currently developing approaches to identify evidence of trans-epigenetic effects by comparing the epigenetic states of parental homozygous TE insertion with the F1 heterozygous TE insertion site. We will also perform transcriptome analysis to test if such trans-epigenetic effects result in trans-silencing. Our study will provide new insight into how the epigenetic silencing of TEs may have inadvertent harmful consequences not only in cis but also in trans. Moreover, if true, this TE-induced trans-silencing should be incorporated to study how TEs shape the evolution of the genome landscape.

650T **Extending the GENCODE long non-coding RNA catalogue in human and mouse** Adam Frankish[1], Toby Hunt[1], Jose Manuel Gonzalez[1], Silvia Carbonell-Sala[2], Gazaldeep Kaur[2], Jonathan Mudge[1], Roderic Guigó[2] [1]EMBL-EBI, [2]Centro de Regulación Genómica

The GENCODE consortium produces detailed reference annotation of all human and mouse protein-coding genes, pseudogenes, long non-coding RNAs (lncRNAs) and small RNAs. Accurate gene annotation is of fundamental importance for genome biology and clinical genomics; annotation that is incorrect or incomplete impacts downstream analysis and introduces potentially significant errors.

As part of the consortium's efforts, the CapTrap-seq protocol has been developed to achieve reliable end-to-end production of long-read RNA sequences using both PacBio and ONT platforms. Combining CapTrap and oligo(dT) priming to detect 5'-capped, full-length transcripts with the Capture Long Seq (CLS) method's targeted capture of putative lncRNA transcription, we have been able to identify more than 100,000 novel human and mouse lncRNA transcripts. These highly accurate transcripts are then integrated into the GENCODE geneset via a manually supervised computational pipeline.

These significant efforts to expand the GENCODE catalogue of full-length lncRNA transcripts in human and mouse support both intra- and inter-species analysis giving additional insights into the biology of lncRNAs.

The GENCODE genesets are the default Human and Mouse annotation used in the Ensembl and UCSC genome browsers and can be downloaded from www.gencodegenes.org.

651T **Mouse Regulatory Regions and Their Alleles and Sequence Variants at MGI and The Alliance** Laurens G Wilming, Sophia Zhu, Paul Hale, Richard Baldarelli, Joel Richardson, Cynthia Smith, Carol J Bult MGI, The Jackson Laboratory

The role of regulatory regions, such as enhancers, silencers, locus control regions, and imprinting control regions, in genetic networks is subject to increasing interest. To gain more insight into the role of these elements in the complex gene expression networks guiding normal development and disease, researchers are targeting regulatory sequences through genetic engineering to study natural variation and disease association. Mouse Genome Informatics (MGI) at The Jackson Laboratory has imported mouse regulatory region data from Ensembl and the VISTA enhancer database to assist the community in their efforts. The more than 365,000 imported regulatory region markers, named as *Rr* followed by a sequential number (*e.g. Rr123456*), are classified into five categories: enhancers, promoters, promoter flanking regions, CTCF-binding sites, and transcription factor binding sites. In addition to these imported putative regulatory region markers, we manually curate regulatory markers in these categories and more (*e.g.* insulators, silencers, ICRs, LCRs, *etc.*) based on reports in scientific publications. Regulatory region alleles are annotated to the coordinates of the associated allele's underlying molecular sequence variation (deletion, point mutation, *etc.*) where possible. The regulatory region markers and associated allele records are available from MGI (**www.informatics.jax.org**) and from the Alliance of Genome Resources (Alliance) (**www.alliancegenome.org**), where the associated allele genome sequence variants are also available. Examples we will discuss include regulatory elements associated with the *Sox10* gene and the *Klr* the *Hox* gene clusters.

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652T **Improving annotation of introgressions in the laboratory mouse using the Collaborative Cross** Sam Ardery[1,2], Anwica Kashfeen[1], Paul Cotney[1], Colton L Linnertz[1], Md Taksir Hasan Majumder[1], Martin T Ferris[1,3], Fernando Pardo-Manuel de Villena[1,4] [1]Department of Genetics, University of North Carolina-Chapel Hill, [2]Program in Genetics and Molecular Biology, University of North Carolina-Chapel Hill, [3]Systems Genetics Core Facility, University of North Carolina-Chapel Hill, [4]Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill

An introgressed sequence is a region of the genome transferred across two divergent taxa and maintained despite repeated backcrossing to the recipient species. This process introduces contiguous regions of a higher-than-average amount of genetic diversity that show an ability to exchange DNA between taxa, or hybridization Analysis of these regions can be used to date this genome exchange and measure the selective forces maintaining the introgression. Introgressions can be identified by scanning the genome for regions with phylogenetic histories that are inconsistent with the global phylogeny. However, this task is complicated by the dynamic nature of introgressed regions as well as challenges with technical analyses of whole-genome sequencing, making analyses of introgressions difficult in outbred populations. The mouse presents an attractive model for these studies for several reasons: their natural species ranges are known, we have extensive and detailed records of laboratory mice since the beginning of their use as a model in classical biomedical research, and we have detailed genetic/sequencing data from both wild and laboratory mouse strains. We also have ample evidence of introgressed regions in both classical inbred laboratory mouse strains, as well as in wild mouse populations. Here, we utilize whole-genome sequencing data from the Collaborative Cross (CC) mouse population to improve our annotation of introgressed genome regions of the mouse genome. We take advantage of our recently described methodology for identification of endogenous retrovirus (ERV) insertions that segregate within the CC population. ERV insertion is a type of mutation which has attributes that are complementary to classical single nucleotide variant (SNV) analyses. As an example, we have already refined a region on Chromosome 14 from 44 Mb to 46 Mb in NZO/HILtJ where the subspecific origin is not the canonical *M.m.domesticus*, but rather *M.m.musculus*, extending this region of introgression in NZO. As such, we can use these insertions to refine the boundaries of classically annotated introgressed regions, identify novel introgressed regions, and also better understand the extent of incomplete lineage sorting between *Mus musculus castaneous*, *M.m. domesticus*, and *M.m. musculus* subspecies of mouse.

653T **Molecular Characteristics and Computational Analysis to Investigate Genetic Diversity and its impact on Post Translational Modifications** Muhammad Saleem University of the Punjab

Various strains of Sordaria fimicola were collected from the south-facing African Slope (AS) and north-facing European Slope (ES) microsites within the Evolution Canyon valley. A comprehensive array of molecular, genetic, and in silico analyses were executed to delineate distinctive genetic markers for the purpose of investigating genetic variations and post-translational modifications. These markers encompassed two non-histone methyltransferases (EFM6 and EFM7), three histone methyltransferases (RKM1, RKM4, and Set 9), and five genes encoding industrially significant enzymes, namely endo-1,4-β xylanase, β-xylanase, carboxypeptidase, dipeptidyl-aminopeptidase (DAPD), and Serine protease endopeptidase (SP2). The

primary objective was to examine whether harsh environment accelerates the occurrence of genetic variations.
In this study, 10-kilobase genomic DNA from Sordaria strains was subjected to amplification, resulting in the identification of 32 nucleotide variations across all strains when compared to the consensus sequence. Furthermore, a total of 30 and 38 potential post-translational modification (PTM) sites, along with 10 and 7 inter-strain polymorphic sites, were predicted for AS and ES strains, respectively. In the case of EFM7, the analysis of genetic variations unveiled 54 sites leading to changes in amino acids at 16 sites spanning 9 positions in the protein sequence. Notably, the investigation predicted 67 potential sites for significant PTMs within the EFM7 domain, which could potentially enhance its catalytic activity.
Moving on to the RKM1 and RKM4 genes, seven single nucleotide polymorphisms (SNPs) were identified in the former and nine in the latter, with a subset of SNPs being unique to AS strains. A parallel trend was observed in SP2, where six polymorphic sites and 35 potential PTM sites were found in AS strains. Similarly, AS strains displayed a higher degree of genetic variation in genes associated with endo-1,4-β xylanase, β-xylanase, carboxypeptidase, and DAPD in comparison to the relatively stable ES strains. In short, this study elucidates that mutations resulting from natural genetic variations under environmental stress conditions gradually reshape the genetic composition of species, particularly in the case of Sordaria fungi at microsites. This research contributes to unraveling the mysteries surrounding the process of evolution.
Keywords: Genetic variations, Sordaria fimicola, EFMs, RKM4, Post-translational modifications (PTMs).

654T **Genome-wide overexpression screens in *Saccharomyces cerevisiae* identify novel antifungal drug-resistance mechanisms** Akriti Agrawal[1], Ina Suresh[2], Chris Jackson[2], David Gresham[2] [1]Biology, NYU, [2]NYU

Fungal infections pose a critical global health crisis, affecting more than a billion people and killing more than a million people annually. A central challenge to treating eukaryotic pathogens is the limited number of fungal-specific drug targets. Identifying the genetic basis of drug resistance is critical for devising effective treatment strategies. Previously, genome-wide studies of alleles that confer resistance to antifungals have used gene deletion collections to identify loss of function that confer resistance. However, the genetic basis of drug resistance in many clinical strains results from gain of function mutations such as copy number variants (CNVs) of the *ERG11* gene, which result in increased gene expression and resistance to fluconazole (an azole drug that interrupts ergosterol synthesis). Therefore, we screened the *Saccharomyces cerevisiae* diploid YETI collection, genome-wide mutation collection in which each gene is inducibly overexpressed, to identify genes which confer antifungal drug resistance when overexpressed. Using a time-series experimental design we quantified the fitness effect of each overexpression allele in the presence and absence of fluconazole. We identified known genes and pathways that result in increased fluconazole resistance, including multiple components of the ergosterol biosynthesis (*ERG11*, *ERG13*) and efflux pathway (*PDR5 and PDR3*). In addition, we identified several previously unknown genes that increase resistance to fluconazole revealing multiple possible mechanisms of resistance, including the oxidative stress sensors *YAP1 and MGE1*. Interestingly, we also identified genes that increase sensitivity to antifungals when overexpressed, such as *AUS1*, a sterol-importing transporter. Extension of our approach to other antifungal drugs will enable identification of common and drug-specific mechanisms of resistance.

655T **How repeats rearrange chromosomes in deer mice** Landen Gozashti, Olivia Harringmeyer, Hopi E Hoekstra Department of Organismic & Evolutionary Biology, Department of Molecular & Cellular Biology, Museum of Comparative Zoology, Harvard University

The origin and evolution of karyotypic diversity has long interested evolutionary biologists and geneticists. Large chromosomal rearrangements, such as chromosomal inversions, often underlie karyotype variability, but the mechanisms by which these rearrangements arise remains poorly understood. The deer mouse (*Peromyscus maniculatus*) is an excellent model system for studying karyotypic evolution because wild populations of deer mice display widespread variation in their number of biarmed chromosomes (indicative of large chromosomal rearrangements), yet a highly conserved total number of chromosomes. Recent work has shown that mega-base scale inversion polymorphisms underlie much of the karyotypic variability found within deer mice, including re-positioning centromeres from acrocentric to metacentric positions, raising the question of how these inversions arise within the deer mouse genome. Here, we investigate the genomic basis of deer mouse inversion polymorphisms. We first employed a combination of long-read (PacBio Hifi) and proximity ligation (Hi-C) sequencing of two F1 hybrid mice to create phased chromosome-level de novo genome assemblies for four deer mouse subspecies. Through comparisons of these genomes, we identified ~8,000 inversion polymorphisms, which together affect over 30% of the genome. We next investigated inversion breakpoints, and found that smaller inversions are often flanked by retrotransposons, suggesting they primarily arose via ectopic recombination between these retrotransposons. In contrast, the breakpoint regions of large (>1Mb) inversions are primarily enriched for segmental duplications (long repeats ranging from kilo- to mega-bases in length). Interestingly, we found evidence that retrotransposons may drive segmental duplication formation, suggesting that retrotransposons may indirectly facilitate the formation of mega-base scale inversions. Furthermore, we were interested in why inversion breakpoints often occur near centromeres. We found that pericentromeric regions exhibit a recent accumulation

of retrotransposons, which likely facilitated an expansion of pericentromeric segmental duplications. By comparing inversion breakpoints as well as fully assembled centromeres between ancestral and derived karyotypes, we highlight the evolutionary processes that give rise to intraspecific karyotypic variation with unprecedented resolution. Together, our results are consistent with a model whereby retrotransposable element activity and distribution directly and indirectly influences mammalian karyotypic evolution.

656T **Assembly and characterization of W chromosome in monarch butterfly (*Danaus plexippus*)** Martina Dalikova[1], Daniel Portik[2], Sarah B. Kingan[2], Hayley Mangelson[3], Liuqi Gu[1], James R. Walters[1] [1]Department of Ecology and Evolutionary Biology, University of Kansas, [2]Pacific Biosciences, [3]Phase Genomics

Degenerated sex chromosomes have a precedent of being notoriously difficult to assemble. Recent advances in long-read sequencing are catalyzing increases in the number and quality of W and Y assemblies, providing novel insights concerning the content and evolution of these elusive chromosomes. Here we report a novel genome assembly for the monarch butterfly (*Danaus plexippus*), focusing on the W chromosome. This species harbors a neo-Z chromosome, arising from the fusion of the ancestral Z with an autosome. Previous cytogenetic analyses indicated a similarly large and bipartite W chromosome, suggesting the possibility of a comparable neo-W, but much ambiguity remains concerning the sequence and history of the monarch W chromosome. We generated PacBio HiFi reads with Hi-C data from females to support *de novo* assemblies of maternal and paternal haplotypes using Trio binning. This produced chromosomal-level scaffolds for the Z and all autosomes. Approximately 14 Mbp of W-linked contigs from the maternal genome were identified based on male-to-female coverage and sex-specific k-mers. The W chromosome scaffold length was around 10 Mbp, thus leaving about one-third of this chromosome in unscaffolded contigs. We used this new assembly to investigate the gene and repeat content as well as population genetic diversity of the monarch W chromosome. The W is highly repetitive and contains very few protein-coding genes, which mainly arose through retroposition or segmental duplication from other chromosomes. Exons of these W-linked copies retain high sequence identity compared to their Z-linked and autosomal counterparts despite substantial divergence in intergenic regions, suggesting strong stabilizing selection on protein-coding regions for these W genes. Moreover, at least some W genes appear to be expressed in female tissues, especially ovaries. The prevalent repetitive content of the W chromosome is formed by transposable elements (TEs) from the LINE and LTR retrotransposon groups. Surprisingly, the TEs on the W chromosome have strikingly lower divergence compared to the rest of the genome, which we suggest results from gene conversion arising from TEs occurring in tandem arrays. Population genetic analyses revealed nucleotide diversity is about ten times lower on the W chromosome compared to autosomes. Finally, despite this novel W assembly, strong evidence for or against a neo-W chromosome origin remains elusive.

657T **Altered hydroxymethylation of imprinted genes in male infertility** Rajender Singh, Sujit Mohanty Endocrinology, Central Drug Research Institute

Methylation of imprinted genes has been correlated with low sperm count and motility, but since methylation analysis cannot discriminate between methylation and hydroxymethylation, the contribution of hydroxymethylation to such correlations remains completely unknown. Changes in hydroxymethylation in male infertility have not been investigated in depth. We undertook the present study to investigate changes in hydroxymethylation in male infertility. We analysed hydroxymethylation of imprinted genes in infertile cases and fertile controls. This was followed by the analysis of DNA methyltransferases (DNMT) and ten-eleven translocation methylcytosine dioxygenases (TETs) transcript and protein levels in cases and controls. In total, this case-control study used spermatozoal DNA, RNA, and protein from 135 oligozoospermic, 45 normozoospermic, and 66 fertile controls, respectively. Imprinting-controlling regions of imprinted genes were analysed for methylation using deep sequencing on spermatozoal DNA from 32 oligo/oligoastheno-zoospermic infertile patients and 20 normozoospermic fertile men. Further, hydroxymethylation analysis of the same imprinted genes was carried out using real-time PCR based on the spermatozoal DNA from 15 Normozoospermic infertile, 31 Oligo/Oligoasthenozoospermic infertile patients, and 15 Normozoospermic fertile. We observed that the hydroxymethylation level in the H19, IGF2, and LIT1 genes was significantly higher, while those of the MEST and PEG3 genes were lower in the cases in comparison to controls. Within the case groups, H19 and LIT1 hydroxymethylation showed direct correlation with sperm count, IGF2 and MEST showed direct correlation with some exceptions, and PEG3 showed inverse correlation. We also analyzed DNA methyltransferases (DNMTs) and found higher expressions of DNMT1 and DNMT3B in the cases in comparison to controls, and within the case group, DNMT1 showed an inverse correlation with sperm count. Analysis of ten-eleven translocation methylcytosine dioxygenases (TETs) showed that TETs were significantly higher in cases in comparison to controls. Within the case groups, TET1 and TET3 increased with an increase in sperm count. In conclusion, aberrant hydroxymethylation of the imprinted genes, i.e., *H19, IGF2, IGF2*, and *MEST,* correlates with reduced sperm count and fertility.

658T **A chromosome scale reference genome of house fly *Musca domestica*** Yesbol Manat[1], Perot Saelao[2], Richard Meisel[1] [1]Biology and Biochemistry, University of Houston, [2]US Department of Agriculture

Sex chromosomes play an important role in the evolution of sexually reproducing species. Studying sex chromosome evolution provides insights into broader principles of evolution, adaptation, and speciation. Investigating relatively young and undifferentiated sex chromosomes is essential for understanding how natural selection, intersexual conflict, and recombination landscapes affect the establishment of new sex chromosomes. The house fly, *Musca domestica*possesses a highly polymorphic sex determination mechanism, where the male-determining locus can be found on multiple different chromosomes. This makes it well suited system for studying the evolution of new sex chromosomes. However, the lack of a chromosome-scale male genome makes it challenging to use the house fly as a model system to study X and Y chromosome evolution. Here, we generated a chromosome scale assembly of *M. domestica* males carrying two different Y chromosomes. We used this chromosome-level genome of *M. domestica* to determine how gene content and recombination rates evolve in shortly after the formation of new sex chromosomes.

659T **Horizontal Gene Transfer in Staphylococcus Epidermis shows evidence of function of phage proteins in the bacteria** Gaurav S Arora, Matthew Thompson Gallaudet University

Horizontal Gene Transfer or HGT is the transfer of genes from one organism to another. HGT can occur between bacteria and bacteriophages. In bacteria, genes received from HGT are quickly removed unless they serve some beneficial purpose.

Prior work has found evidence of HGT of a tail tape measure protein (TMP) in bacteria. TMP occurs in tailed phages and controls the tail length of the virus.*Staphylococcus epidermis* or *S. epidermis* is a ubiquitous inhabitant of human skin and mucous membranes. Originally *S.epidermis* did not cause infections but over the years the number of infections caused by *S. epidermis* have increased.The phage that infects *S.epidermis* belongs to the *Caudovirales* order.

Using Bioinformatics tools, we show evidence of transfer of the TMP in *S. epidermis.* We also see that the protein is conserved in several strains of *S. epidermis.* RNA-seq data from published studies, show that the TMP is expressed in several of these strains.

By gaining the knowledge of conserved genetic information, further insight into the evolution of *S.epidermis* are gained. Also, it will lead to further understanding of the interaction between virus and bacteria. It may also help understand the changes that may have caused the pathogenic nature of the bacteria.

660T **Long-read *de novo* assembly and comparative analysis of six howler monkey genomes within genus *Alouatta*** Bide Chen[1], Patrícia Domingues de Freitas[2], Luana Portela[2], Victor Yunes[3], Lilian Catennacci[4], Alcides Pissinatti[5], Amy Goldberg[1] [1]Evolutionary anthropology, Duke University, [2]Federal University of São Carlos, [3]São Paulo State University, [4]Federal University of Piauí, [5]Primatology Center of Rio de Janeiro

As our closest relatives, primates are an ideal system to understand human evolution, and mechanisms generating genetic variation broadly across animals. Though only one species in the genus *Homo* exists today, multiple monkeys have wide radiations, allowing us to compare closely related species within a genus. Despite a growing number of primate genomes available, most genera are represented by a single reference genome. Here, we generate *de novo* reference assemblies using long-read sequencing for 6 Howler monkeys (genus *Alouatta*) to understand within-genus, between-species evolution. With between 9 to 14 species howler monkeys are some of the most widely distributed platyrrhines, extending from southern Mexico to northern Argentina, inhabiting a range of environments. Prior phylogenetic investigations into howler monkeys have exhibited discrepancies across studies or remained inconclusive, based on a mix of cytogenetic, morphological traits, and a restricted set of molecular markers. Attempts to address these questions have been limited because only a single poor-quality reference genome of mantled howler monkey (*Alouatta palliata*, Mexico) is currently available (assembly contig count: 1152695; N50: 51.304 Kbp). Our assemblies contain roughly ~2000 contigs, with N50 of ~15Mb and L50 of ~60. Based on phylogeny constructed with OrthoFinder, IQtree, and ASTRALL, we find the clade grouping *A. seniculus* and and *A. guariba* as sister taxa, with *A. caraya* and *A. belzebul* as respective sister lineages to this clade. We identify loci that show species-specific signatures of selection, as well as adaptive loci shared across the genus.

661T **Characterization of the Structure and Stability of Wild-Type FGF2 and Mutant Design of Wild-Type FGF2 to Enhance Wild-Type FGF2 Stability** Aisha Al-Rizzo, Suresh Thallapuranam, Patience Okoto Chemistry and Biochemistry, University of Arkansas

FGF2 is a multifunctional growth factor, part of the highly conserved superfamily of fibroblast growth factors. FGF2, along with other growth factors, belongs within a vast family of polypeptide growth factors which exhibit mitogenetic and cell proliferative properties. In the process of embryonic development, some fibroblast growth factors can play numerous roles in cell proliferation, migration, and differentiation. Other FGFs can present as homeostatic factors in adulthood, which help function in tissue repair, angiogenesis, and injury response, while other factors can present in embryonic development. FGFs

accomplish such biological and pathophysiological processes via paracrine, intracrine or endocrine signaling. FGF2 acts through tyrosine kinase receptors and exhibits cell self-renewal, cell differentiation and migration, wound healing, and angiogenesis, important properties in upkeeping homeostasis within the body. In particular, FGF2 confers its functions through paracrine signaling. FGF2 is found in embryonic and adult cell types, plays a significant role in the development and function of multiple organ systems. Furthermore, FGF2 plays roles in angiogenesis (blood vessel formation) and wound healing, which shows promise in medical applications and tissue engineering. Although promising in its potential, wild-type FGF2 itself poses issues of inherent instability, which affect its overall function. In our group, we are designing mutants of wild-type FGF2 using single point mutations to enhance FGF2 stability. By eradicating this issue, a higher stability in FGF2 can be applied to biochemical and medical industries, to name a few. For example, thermal instability wouldn't be an issue with a more stable FGF2 protein when considering external factors, such as the supply chain when transporting for further biochemical research. Thermal stability also indicates a longer shelf life, if used in medical applications. Overall, the aim of this project is to improve the stability of wild-type FGF2, a potent but unstable fibroblast growth factor, thus improving its function via experimentative methods of small-scale and large-scale expression, purification, and characterization of structure and stability. In short, the goal of the experimental design is to compare cell proliferation activity of wild-type FGF2. The ability to solve the problem of instability in FGF2 by fabricating a more stable and functional growth factor can open new doors for further research and breakthroughs in the fields of biochemistry and medicine.

662T **Unexpectedly simple and powerful relationships exist between genome composition and DNA Methylation, mRNA and Protein Expression in *Arabidopsis thaliana* rosettes** Richard Mott[1], Ziming Zhong[2], Mark Bailey[3], Yong-In Kim[4], Briony Parker[3], Nazanin Pesaran-Afsharyan[3], Kirsty Hassell[3], Gancho Slavov[5], Keywan Hassani-Pak[3], Kathryn Lilley[4], Frederica Theodoulou[6] [1]University College London, [2]Genetics, Evolution and Enviroment, University college London, [3]Rothamsted Research, [4]Dept Biochemistry, University of Cambridge, [5]IBERS, University of Aberwrystwyth, [6]Plant Sciences and the Bioeconomy, Rothamsted Research

The variation in expression across the proteome and transcriptome from a single plant tissue varies by over four orders of magnitude, despite most nuclear genes being encoded only once. The generally accepted explanation is that the observed expression of a given transcript reflects a specific balance between the rates of transcription and degradation, and that the expression of the corresponding protein is further modulated by a similar balance between translation and turnover. We set out to understand how much of variation of these omic levels within a single tissue can be explained by simple genomic features when genetic and environmental variation is absent. Specifically we investigated the impact of gene-body CpG methylation (gbM) and codon composition on these higher omic levels.

We performed detailed genomic profiling of the two *Arabidopsis thaliana* accessions Col-0 and Can-0. We re-assembled their genomes using long reads, from which we also calculated baseline gbM for each gene, and measured rosette gene and protein expression in multiple biological replicates grown under tightly controlled conditions, and which produced highly reproducible results.

We re-annotated the gene and transposon content of each assembled genome to produce accurate data across omic levels. We fitted models to explain variation in methylation, gene and protein expression in terms of lower omic levels. We find that the single best predictor of any omic level in one accession is to measure the corresponding level in the other accession, despite their 0.5% sequence divergence, and the presence of many differentially expressed genes. Looking within an accession, the impact of baseline gbM on either mRNA or protein expression can be almost entirely explained by variation in that gene's codon composition. Thus, absent environmental perturbation, gbM is determined by local sequence features.

We also find very similar impacts of any given codon on both mRNA and protein composition. These impacts are highly significant and unrelated to genome-wide codon abundance. About 44% of the variation in protein expression is explained by a combination of mRNA and sequence composition, each contributing distinct information. Codon composition alone explains 27% of protein expression and 44% of transcript variation. These statistics suggest that these simple models are important, but by no means the only, drivers of expression variation.

663T **Comparative genome analysis of *Quercus rubra* and *Q. ellipsoidalis*** Swapan Chakrabarty[1,2], Carsten Külheim[1] [1]College of Forest Resources and Environmental Science, Michigan Technological University, [2]College of Computing, Department of Computer Science, Michigan Technological University

Oaks (*Quercus spp.*) are the most abundant hardwood tree genus in the Northern Hemisphere, and they are widely distributed in the Americas, Asia, Europe, and North Africa. The rapid change in climatic and edaphic factors pose severe threats to the growth and survival of oak populations. Northern red oak (*Quercus rubra*) has a broad geographic distribution in easter North America and thrives on mesic loamy soils. In contrast, northern pin oak (*Q. ellipsoidalis*) has a narrower geographic

distribution in the Midwest of the U.S.A. and prefers more xeric, sandy sites. These related, and frequently hybridizing, species provide a model for adaptation to drier environments. Samples of *Q. ellipsoidalis* were collected from a single tree for library construction and sequencing. The whole genome *Q. ellipsoidalis* has been sequenced with PacBio long reads, Illumina short reads and Hi-C sequencing. Twelve different types of tissues were used for RNA sequencing. The BUSCO genome completeness is 98.0% with N50 of 61,697,690 bp. In this study, we compared the genome structure of *Q. ellipsoidalis* with *Q. rubra*. Synteny analysis revealed the preserved co-localization of the chromosomes between these species. Sequencing of *Q. ellipsoidalis* genome has made it possible to analyze structural variants (SVs) on a massive scale, which further gives the required knowledge to investigate SV development mechanisms and to investigate their functional impact. The genome of *Q. ellipsoidalis* provide strong fundamentals of understanding the adaptation of oaks in the environment. Our study not only introduces new genomics resources for *Q. ellipsoidalis* research but also exemplifies how structural variation drive fundamental functional discoveries in oak adaptation. We further investigated and compared the gene content of gene families related to root formation and drought adaptation.

664T **Improvements of *Xenopus* genome annotations for the Alliance of Genomic Resources and other Amphibian genomes** Taejoon Kwon[1], Malcolm Fisher[2], Christina James-Zorn[2], Andrew Bell[2], Virgilio Ponferrada[2], Ngoc Ly[2], Sergei Agalakov[3], Brad Arshinof[3], Stan Chu[3], Kamran Karimi[3], Vaneet Lotay[3], Troy Pells[3], Dong Zhuo Wang[3], Peter Vize[3], Aaron Zorn[2] [1]Biomedical Engineering, Ulsan National Institute of Science and Technology (UNIST), [2]Cincinnati Children's Hospital Medical Center, [3]University of Calgary

Two representative genomes of *Xenopus* species (*X. laevis* and *X. tropicalis*) have been completely sequenced recently. However, their genome annotations are still challenging, especially due to different versions being able from different sources (NCBI RefSeq, EnsEMBL, and JGI/UCB). Here, we present how we have integrated these widely used annotations, including their historical builds for the older version of genome sequences, to make comprehensive reference annotations for these species. To evaluate the accuracy of gene model predictions in each method and to resolve the conflict when this happens, we have developed a data-driven evaluation method that utilizes the publicly available RNA-seq and other functional genomic resources. Additionally, we compared these results to other reference species participating in the Alliance of Genomic Resources consortium so we could access higher levels of genomic resource analysis, such as phenotypes related to human disease modeling. Our efforts to improve the annotation of these two *Xenopus* reference genomes are also a valuable resource to improve the annotations of other recently sequenced amphibian genomes, not only for the other Pipidae, like *H. boettgeri*, *X. borealis*, and *X. petersii*, but also other amphibian genomes such as salamanders (*A. mexicanum* and *P. waltl*) and caecilians (*G. seraphini* and *M. unicolor*).

665T **Genetic analysis of the yeast DNA damage response with a genome-wide inducible degron library** Eduardo Gameiro[1], Jia Jun Fung[1], Brian Luke[1,2], Anton Khmelinskii[1] [1]Institute of Molecular Biology gGmbH, [2]Johannes Gutenberg University

The yeast knockout library, comprised of strains carrying deletions of individual open reading frames (ORFs), has been a vital tool to study gene function through both classical and high-throughput approaches over the last two decades. However, the knockout library is limited to non-essential genes, and must be complemented with conditional or hypomorphic alleles of essential genes for complete genome-wide studies. Furthermore, errors in strain annotation, combined with the continuous generation of spontaneous suppressor mutations, can lead to mistakes in functional annotations. To circumvent these problems, here we describe genome-wide libraries of degron alleles for inducible protein depletion.

We constructed two libraries with over 5600 essential and non-essential genes tagged with an auxin-inducible degron (AID*), and an optional fluorescent protein (FP), at the C-terminus of each ORF. The FP tag allows assessing the degradation efficiency in high throughput but substantially increases the overall tag size, with a greater chance to affect protein function. Using fluorescence measurements, we found that approximately 80% of the strains exhibited complete or partial degradation of the tagged protein in the presence of auxin. Partial degradation was more frequent for highly expressed proteins, suggesting saturation of the AID machinery. Furthermore, the efficiency of protein degradation varied between cellular compartments, and was lowest for mitochondrial, vacuolar, Golgi and ER proteins, likely due to tag accessibility. Using the non-fluorescent library, we show that over 60% of tagged essential protein alleles display a fitness defect upon induction of protein degradation. Using these libraries, we screened for DNA damage response factors. Viability screens with hydroxyurea, camptothecin and methyl methanosulfonate (HU, CPT and MMS respectively) identified essential protein alleles that are sensitive to these DNA damage drugs, opening the possibility to discover new gene functions that could not be tested with previously established libraries. Taken together, our work shows that the inducible degron yeast collection is a valuable tool to study gene function, providing mutants with a conditional and fast protein depletion, which can facilitate systematic characterization of the yeast genome.

666T **Characterization of Type II-A Anti-CRISPR Genes** Dinie Zheng, Meru J Sadhu National Human Genome Research

Institute, NIH

CRISPR-Cas systems are found naturally in bacteria as defense systems against bacteriophages. Phages have in turn evolved to have anti-CRISPRs, which are small proteins that inhibit CRISPR-Cas systems. Currently, there have been about 100 anti-CRISPRs that have been discovered, 32 of which specifically target type II-A CRISPR-Cas systems. Many anti-CRISPRs still remain to be discovered and elucidated for their mechanisms. We will seek to characterize over a thousand type II-A anti-CRISPR genes by utilizing large scale oligonucleotide synthesis to synthesize these genes to test for their ability to inhibit type II-A CRISPR-Cas systems in yeast. This research will contribute towards the understanding of anti-CRISPR function and expand upon the arsenal of existing anti-CRISPR genes.

667T **Unveiling the spectrum of potential hosts for SAR-CoV-2 using a high-throughput yeast display screen.** Mudabir Abdullah, Michael Chambers, Sophie Scobell, Meru Sadhu NHGRI, National Institute of Health

The global impact of SAR-CoV-2 extends beyond humans, raising concerns about potential reservoirs and broader implications for disease transmission in various animal species. Understanding the virus's behavior necessitates a comprehensive assessment of its potential host range. Central to this inquiry is the angiotensin converting enzyme 2 (ACE2), the primary attachment site for SARS-CoV-2 spike protein, which exhibits substantial variability among species. The ability of the spike protein to interact with ACE2 receptors in potential animal hosts emerges as a pivotal determinant for host compatibility. In this study we use a high-throughput assessment of ACE2 compatibility with coronavirus spike proteins across hundreds of diverse animal species. The interface of ACE2 with the spike protein exhibits high amino acid diversity between species, influencing the potential binding of spike orthologs. Previously it was shown that the main spike interacting region on the surface of ACE2 involve amino acids from 24-83 and 329-363. To elucidate these interactions, we constructed a library of chimeric ACE2 receptors representing various species orthologs in the ACE2 region crucial for interfacing with SAR-CoV-2 spike protein. We printed an oligo array of DNA molecules containing these segment ACE2 orthologs representing 821 species and clone them into a backbone of the human ACE2 gene. The chimeric ACE2s libraries are transformed into yeast expressing the ACE2 protein on its surface.

These chimeric ACE2 receptors are expressed via yeast surface display and subsequently incubated with fluorescently labeled SAR-CoV-2 spike proteins. The spike-binding ACE2 chimeras are enriched through fluorescence-activated cell sorting (FACS) and identified through high-throughput Illumina sequencing. This approach enables the exploration of whether SARS-CoV-2 variants have altered host range, offering insights into the future dynamics of SAR-CoV-2 host compatibility. This investigation is pivotal in discerning whether the host range will remain consistent or evolve into a subset of the original potential hosts.

668T **Chromatin Structure in the *Ogataea polymorpha* Species Complex** Tiffany Lundberg[1], Daniel Turevski[2], Andrew Klocko[1], Sara J Hanson[2] [1]University of Colorado-Colorado Springs, [2]Colorado College

Three-dimensional chromosome organization and chromatin structure are critical to the regulation of eukaryotic genome function. In budding yeasts, the mechanism for heterochromatin formation underwent a transition in which the Clr4-mediated histone H3 lysine 9 methylation mechanism found in multicellular eukaryotes and the fission yeast *Schizosaccharomyces pombe* was lost and the expanded Sir2 histone deacetylation mechanism observed in *Saccharomyces cerevisiae* was gained. However, the mechanism for heterochromatin formation in yeast species that diverged after the loss of Clr4 heterochromatin formation but prior to the expansion of the Sir2 pathway are less understood. One such species is the methylotrophic yeast, *Ogataea polymorpha*. To gain insights into chromatin structure of constitutively heterochromatic and euchromatic regions of the genomes of two members of the *O. polymorpha* species complex, we used chromatin immunoprecipitation-sequencing (ChIP-seq) to examine histone post-translational modifications and chromosome conformation capture coupled with high-throughput sequencing (Hi-C) to assess three-dimensional genome organization in two members of the *O. polymorpha* species complex.

669T **Educational Resources Hosted at the *Saccharomyces* Genome Database** Rob Nash[1], Suzi Aleksander[1], Marek S. Skrzypek[1], Jodi Lew-Smith[1], Rahi Navelkar[2], Edith D. Wong[1], Stacia R. Engel[1], J. Michael Cherry[1], The SGD Project[1] [1]Genetics, Stanford University, [2]Department of Biomedical Informatics, Harvard University

The *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org) is the leading community resource for the budding yeast *S. cerevisiae*. SGD provides high-quality, manually curated information on the yeast genome and offers a wide variety of tools and features that make it an indispensable resource for researchers. SGD engages in a variety of online training and educational outreach efforts to inform our user community about new developments, to improve familiarity with SGD features and tools, and to increase public awareness of the importance of yeast not only for biological and biomedical research but also for instructional purposes.

The SGD community wiki (https://wiki.yeastgenome.org) provides users with a venue for accessing and sharing information in areas that include educational resources. This includes information about associations and societies, general and yeast specific classroom materials (teaching modules and project-based courses), and some fun sites of general interest to the aspiring biologist.

To inform the community about new features and tools, SGD creates and posts short videos to YouTube (https://www.youtube.com/SaccharomycesGenomeDatabase) as a means to both educate our users and address their questions. This includes videos on how to use tools like: YeastMine, Variant Viewer, GO Term Finder, GO Slim Mapper and JBrowse, as well as videos to support users interested in navigating phenotypes, interactions, expression data, literature, homologs, human-disease connections and functional complementation.

SGD is also working with micropublications.org to promote the publication of brief, novel, technically sound research results and data that don't fit into full-length articles. This includes single high-quality research results as well as negative results that will accelerate scientific discovery and advance the scientific endeavor. This mechanism for publication is particularly attractive for students interested in rapidly publishing findings of general interest to the greater scientific community. Micropublications are indexed at PubMed, PubMed Central and Europe PMC for greater visibility.

We will continue to develop these services to provide access to educational resources and outreach for students, teachers, and scientists to facilitate greater use and understanding of the resources made available by SGD. This work is supported by a grant from the NHGRI (U41 HG001315).

670T **Transcription associated mutations in haploid and diploid yeast** Rutuja M Gupte, Nathaniel Sharp Genetics, University of Wisconsin-Madison

The highly transcribed regions of the genome are thought to be more susceptible to mutations. Such mutations are called transcription associated mutations (TAM) which can occur through multiple mechanisms. Strand separation due to helicase action during transcription exposes the non-transcribed strand and introduces the possibility of the formation of a 3 stranded R loop in the non-transcribed strand which is highly vulnerable to damage or formation of secondary structures. Processes such as supercoiling promote R loop formation. Transcription associated recombination is also a possibility when there is a head-on or co-transcriptional collision between DNA and RNA polymerase. Head-on collisions, which are more detrimental, occur on the lagging strand.

TAM results in rapid changes in the genome. A positive correlation has been observed between transcription and mutagenesis in introns. These mutations are associated with DNA damage, action of topoisomerase 1, RNA-DNA hybrids, T to U base conversions, stress etc. While these processes persist in bacteria as well as higher eukaryotes, the present study focuses on TAM in yeast.

We are studying the mutation and replication data from ancestor and mutation accumulation lines from a mutation accumulation experiment in haploid and diploid *Saccharomyces cerevisiae*. We are combining mutation, replication and transcription data and also making comparisons between haploid and diploid lines. This study will utilize the available Nascent Elongating Transcript Sequencing (NET Seq) data of the strains used that captures differential transcription rates for rapidly degrading transcription products. This sequencing method uses the stability of the DNA-RNA-RNAP ternary complex and involves sequencing of transcripts from the 3' end. Preliminary analysis of this data indicates that in haploids, which have a higher SNM rate, SNMs are associated with a higher transcription rate which is not true for diploids. Indels are associated with higher transcription rates in both cell types.

This study will provide insights into the repair mechanisms and their effectiveness in the highly transcribed regions, present a comparative analysis of transcription and mutations in coding as well as non-coding regions of the genome and can shed light on the variation in mutation detection across the genome.

671F **Genetic Interactions Between the Gut Microbiota and *C. elegans* Intestinal Cells** Jessica Hill[1], Andrew Moore[2], Justin Ellis[3], Sabrina Zhi[4], Izabella Mastroianni[5], Erin Nishimura[3] [1]BMB, Colorado State University, [2]BMB, Colorado Sate University, [3]BMB, Colorado state university, [4]University of Guam, [5]NIAID

The human gastrointestinal tract is home to trillions of microorganisms which dynamically comprise our gut microbiome. Alteration to our gut microbial community leads to disease. At the forefront of host-microbe interactions are intestinal cells, which establish and maintain beneficial symbiotic relationships with our gut microbiota. *However, how intestinal cells differentiate between symbionts and non-symbionts is unknown. Furthermore, which intestinal cell responses promote symbiont selection in the gut is unclear.* To understand how the intestine responds to distinct bacteria and bacterial

communities, we first generated transcriptional profiles of the whole intestine for *Caenorhabditis elegans* exposed to *E. coli* OP50 (standard lab diet), *Pseudomonas aeruginosa* PA14 (pathogen), and the CeMbio community (simplified, curated microbiome). We found that nearly 5,300 genes differentiate L4 worms exposed to the CeMbio community versus OP50 (2365 up and 2966 down regulated). Notably, pathways involved in muscle regulation and cytoskeletal processes were upregulated in worms exposed to CeMbio compared with OP50 exposed worms, while energy production and biomolecule synthesis pathways were downregulated. Interestingly, worms exposed to CeMbio prior to challenge with the pathogen PA14 displayed less dynamic changes in total gene expression (3.2% increase and 5.4% decrease) compared with worms exposed to OP50 prior to PA14 challenge (6.7% increase and 9% decrease). This is suggestive of a protective effect by the CeMbio community. Next, to characterize the baseline patterns of intestinal colonization for OP50 and the CeMbio community, we made fluorescent reporter strains of each. We found that live bacteria could be observed throughout the intestine, but that both OP50 and the CeMbio community shared a preference for the mid to posterior regions of the intestine. Suggesting that these regions offer a more hospitable yet non-selective environment for hosting bacteria. Future work will investigate the gene regulatory networks that either encourage or discourage colonization of bacteria in specific intestinal regions. Overall, these data begin to explain how intestinal cells interact with and respond to gut bacteria. Reciprocally, they also shed light on how different bacteria and bacterial communities respond to intestinal outputs.

672F **Elucidating the population genetics of structural variants with 79 chromosome-level long-read genomes of Drosophila melanogaster** James Hemker[1], Bernard Kim[2], Paul Schmidt[3], Dmitri Petrov[2] [1]Developmental Biology, Stanford University, [2]Biology, Stanford University, [3]Biology, University of Pennsylvania

With recent advancements in long-read sequencing, it is now possible to sequence and assemble extremely high-quality, long-read Drosophila genomes at an affordable price and with limited work, such that one lab can generate many assemblies. We used Oxford Nanopore Technology and Illumina sequencing to generate chromosome-level, hybrid assemblies of 79, highly-inbred *Drosophila melanogaster* strains from Pennsylvania. We leveraged our de novo assemblies to find large (>35bp), novel structural variants (SV's) that are otherwise missed due to reference-mapping biases. SV's have been largely overlooked and poorly studied, especially in non-human population genetics, and existing studies have primarily relied on reference-based SV mapping. We present population genetic analyses on insertions, deletions, inversions, duplications, and TE events, as well as experimentally-validated case studies on adaptive SV's with large effects on fitness and phenotype.

673F **Effects of Nickel on the Gut Microbiome of *Drosophila melanogaster* in multiple genetic backgrounds** Jesse D Petahtegoose, Allie Hutchings, Thomas Merritt Natural Sciences, Laurentian University

The gut microbiome, GMB, community, the suite of microbes inhabiting a digestive system, reflects the host diet and physiology and in many ways can be considered a host phenotype. Interactions are, however, bidirectional with metabolites and metabolic byproducts shared with and between the microbiome and the host. The host genotype and sex impacts physiology and metabolism, defining the environment the GMB finds its collective self in, and may, therefore, also affect the GMB community structure. Results from other labs are, however, split with some studies finding an impact of host genotype and sex on community structure and others concluding that community structure is determined solely by environmental factors. This study is directly testing the impact of sex and genetic background on GMB community variation driven by nickel toxicity using the Drosophila melanogaster system.

A previous study in our lab showed that exposure to a nickel contaminated diet impacted the D. melanogaster GMB community. Interestingly, results indicated an interaction effect between sex Ni concentration on the community structure, suggesting that the impact of sex is likely to be complex. This early study only included a single line of flies, so the potential impact of genetic background was not tested. A current experiment is testing the impact of nickel across multiple genetic backgrounds and both sexes.

How substantial is the relationship between the host and its GMB, i.e. how much of an effect does one have over the other? In addition to a better understanding of the complexity of GMB changes in response to nickel stress, we will further examine the relationship between GMB and host physiology and metabolism. In part, we will explore this relationship by reconstructing the Ni GMB composition in flies that have not been exposed to nickel and assaying for fly physiological responses. Across a range of phenotypic tests do flies with a Ni microbiome mimic the phenotypes of true nickel exposed flies? Through these experiments, we are not suggesting that the microbiome changes are the main drivers of the phenotype changes from nickel exposure, but we are attempting to find the relationship between host and microbiome.

674F **Epigenetic Control of DNA Replication During Early Drosophila Development** Karla I Troncoso, Aaron T Crain, Robert J Duronio Biology, University of North Carolina at Chapel Hill

To duplicate large genomes during the short cell cycles characteristic of early animal development, cells initiate replication

at thousands of origins throughout the genome. Although the machinery and regulatory mechanisms that govern DNA replication are conserved across eukaryotes, metazoan genomes are distinguished by not using DNA sequence to specify origins of replication. Rather, origin specification and replication initiation is thought to be controlled by the local chromatin environment, which is largely modulated by post-translational modifications (PTMs) on histone N-terminal tails and the interplay between the proteins that establish and bind to these modifications. The 20th lysine in histone 4 (H4K20) can be mono-, di-, or tri- methylated. H4K20 monomethylation (me) has been implicated in DNA replication by analyzing mutations of the enzyme responsible for establishing this mark, SET8. A limitation to this approach is that SET8 has non-catalytic functions and non-histone substrates. Consequently, phenotypes observed from the perturbation of SET8 cannot be solely attributed to the loss of the histone PTM. To delineate the individual contributions of SET8 and the H4K20me mark we engineered a single arginine-to-proline substitution mutation within the highly conserved catalytic domain of SET8 that is predicted to render the enzyme catalytically inactive. Drosophila homozygous for this mutation ($SET8^{RP}$) exhibit full viability, unlike a null allele which causes lethality during larval development. While $SET8^{RP}$ females are able to lay eggs, the embryos never hatch. This phenomenon is called maternal effect lethality and indicates a defect in the first phase of embryogenesis, which is governed exclusively by maternally deposited mRNA and protein and characterized by rapid and synchronous cycles of DNA replication and mitosis that occur in a syncytium and exert substantial demands on the process of replication. We found that embryos from $SET8^{RP}$ mothers exhibit severe morphological defects, developmental delays, and defects in DNA replication. Surprisingly, this essential role for SET8's catalytic activity in S-phase progression appears to be independent of the establishment of the H4K20me mark.

675F **What Role Does Brain Tumor Gene, brat, in Drosophila melanogaster Play In Hybrid Dysgenesis from P-element Transposition** An T Bui, Erin Kelleher University of Houston

Transposable elements (TEs) are parasitic-acting DNA that move, causing DNA damage and mutations that lead to genome instability and cell death. *P*-elements in *Drosophila melanogaster* are widely studied, and in an uncontrolled state, DNA damage resulting from *P*-element mobilization causes a sterility syndrome called hybrid dysgenesis. Hybrid dysgenesis occurs when a naive female mates with a male carrying *P*-elements, resulting in sterile offspring. The degree of dysgenic germline loss depends on the activity of *P*-elements, which in turn is determined by host cofactors required for the transcription and translation of *P*-element transposase. However, these host cofactors are rarely known for *P*-elements or other TEs. Previously in the lab, QTL mapping of natural variation in dysgenic sterility identified a QTL peak that includes *brat*. Brat is an RNA binding protein that determines the differentiation of cystoblasts in the female germline via translational repression of Mad and Myc. Since *P*-elements transpose predominantly in the premeiotic germline, including cystoblasts, Brat could regulate transposition directly or could determine the response of germline cells to the harmful effects of transposition. To differentiate between these alternatives we tested dysgenic sterility in multiple *brat* alleles, compared the transposition rate of *P*-elements via a singed weak assay, and quantify the splicing of *P*-elements and its isoforms with Reverse-Transcriptase Quantitative PCR(RT-qPCR). In a dysgenic cross, heterozygotes for multiple *brat* alleles exhibit higher levels of sterility compared to their wild-type siblings, indicating that *brat* function may be a repressor of *P*-elements or dysgenic sterility. In contrast, from the excision assays, *brat* shows no significant effect on *P*-element transposition. However, results from RT-qPCR data may shed some light on the contrasting results between the dysgenic data and the excision assays. Collectively, these findings will deepen our understanding on how host cofactors interact and regulate its parasitic TE invasions.

676F **Histone 4 Lysine 20 mutation Increases Mitotic Recombination in Drosophila** Priscila Santa Rosa[1], Jeff Sekelsky[2], Bob Duronio[2] [1]Genetics and Molecular Biology, University of North Carolina at Chapel Hill, [2]University of North Carolina at Chapel Hill

Mitotic recombination (MR) is an important DNA repair mechanism; however, in some instances, it can lead to loss of heterozygosity (LOH) or chromosomal rearrangements. LOH is believed to be one of the first genetic events to initiate cancer, and it has been linked to cancer progression and aggressiveness. Although LOH via MR is a hallmark of many types of cancer, the biological mechanisms responsible for MR are not completely understood. MR occurs in a context of chromatinized DNA which is regulated by post translational modifications (PTMs) of histone proteins. These histone PTMs are very dynamic and play important roles in genomic processes, however, it is unknown whether the chromatin state dictated by these histone PTMs affects MR frequency. One such histone PTM is the methylation of histone 4 lysine 20 (H4K20) which is involved in several biological processes, such as DNA replication, gene expression/repression, DNA repair, and chromatin compaction. H4K20 is mono-methylated by PR-Set7/Set8 in humans and flies and can be further modified by SUV4-20H1 (di-methylated), and SUV4-20H2 (tri-methylated) in humans; however, in flies Hmt4-20 performs both di and tri-methylation. In Drosophila, mutation of the H4K20 residue to prevent its PTM or the knockout of its mono-methyltransferase, Set8, increases DNA damage in late-replicating regions of the genome leading to cell cycle arrest. Additionally, in human cells the di-methylated form of H4K20 is essential for DNA repair and DNA repair pathway selection. Using *Drosophila melanogaster* and modern molecular

techniques, this project will test how changes in chromatin structure resulting from loss of H4K20 PTM affect MR events. Preliminary data show that flies heterozygous for H4K20R or H4K20A mutation had a significant increase in MR events when compared to wild-type flies (WT). Because MR events in H4K20 mutants occurred in pericentric regions, I am now performing DNA sequencing to test the hypothesis that these events occurred within heterochromatin. This project will provide insights into how chromatin state plays a role in MR events leading to LOH and potentially advance cancer treatment research.

677F **High-quality annotation of model organism genomes from NCBI's RefSeq project** Shashikant Pujar, Tamara Goldfarb, Alex Astashyn, Olga Ermolaeva, Diana Haddad, John Jackson, Vinita Joardar, Vamsi Kodali, Kelly McGarvey, Mike Murphy, Anjana Raina, Craig Wallin, David Webb, Francoise Thibaud-Nissen, Terence Murphy National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health

National Center for Biotechnology Information's (NCBI) RefSeq project aims to provide a comprehensive, high-quality, non-redundant set of annotations, including transcripts, proteins and genomic DNA. RefSeq provides annotation of model organisms included in the Alliance of Genome Resources (AGR), including annotations for mouse, rat, zebrafish, and Xenopus generated through a combination of curation and NCBI RefSeq's Eukaryotic Genome Annotation Pipeline (EGAP). Expert curation includes review and refinement of structural annotation of known RefSeqs (NM_, NP_, NR_ accessions) as well as descriptors for transcripts, proteins and genes. Much of the curated content is based on user-provided data archived in NCBI's databases, data from external databases and from peer-reviewed publications. Curators in the RefSeq group are also involved in targeted annotation of specific genes and gene-families in collaboration with subject experts. Additionally, we provide specialized sequence products to facilitate basic and applied research. The Consensus coding sequence (CCDS) collaboration provides a core set of high-confidence coding regions for human and mouse. RefSeq Select is a dataset containing a representative transcript for all genes in human, mouse and rat, which is useful in applications that require only one transcript per gene. Overall, curation results in high quality annotation and metadata, which in turn informs future automated annotations as well as better ortholog calls. This presentation will provide data on the high-quality automated annotations of Alliance genomes and provide examples of how curation improves whole genome annotation. This work is funded by the National Center for Biotechnology Information of the National Library of Medicine, National Institutes of Health.

678F **Drosophila as a model for precision toxicology** Shannon Smoot[1], Tank Eisman[1], Sophie Fleck[1], Thomas Kaufman[1], John Colbourne[2], Joe Shaw[1], Brian Oliver[3], Jason Tennessen[1] [1]Indiana University, [2]University of Birmingham, [3]NIDDK, NIH

Global industries are rapidly producing and releasing tens of thousands of chemicals, yet the effects of these molecules on environmental and human health are inadequately understood. This lack of knowledge, coupled with current mammalian testing methods that are both expensive and time-consuming, leaves a dangerous knowledge gap that must be addressed using inexpensive and high-throughput models. The fruit fly *Drosophila melanogaster* has emerged as an ideal system for studying the mechanisms by which individual chemicals alter animal behavior, physiology, metabolism, and gene expression. In this regard, *Drosophila* studies are uniquely situated to quickly identify the molecular targets of individual toxicants via the use high-throughput multi-omics and an unparalleled genetic toolkit. Here we demonstrate the power of this approach by analyzing adult male and female flies fed acute doses of sodium arsenite. By using a combination of transcriptomic and metabolomic methods, we identified a series of dose-dependent effects on metabolic pathways and gene expression networks. Our analysis revealed that sodium arsenite exposure not only activates metabolic and stress-related pathways that are known to protect flies against the toxic effects of this metal, but we also uncovered previously undescribed sex-specific responses. Notably, we observed significant changes in the expression of genes involved in oogenesis and seminal fluid protein expression, indicating that sodium arsenite significantly affects gamete formation and function. Consistent with this observation, we demonstrate that both males and females fed sodium arsenite exhibit decreased fecundity and morphological changes in both the ovary and male reproductive system morphology. Overall, our findings demonstrate how *Drosophila* can serve as a powerful model to identify the sex-specific genetic and metabolic response to toxicant exposure when applying a multi-omics approach.

679F **Functional analysis of candidate Drosophila genes involved in aged host responses to Flock House virus infection** Madelyn Buhl[1], Lakbira Sheffield[2], Stanislava Chtarbanova[3] [1]Biological Sciences, The University of Alabama, [2]University of Alabama Birmingham, [3]University of Alabama

Compared to young adults , aged individuals exhibit greater susceptibility and higher mortality rates to infections caused by viruses, as highlighted by the recent COVID-19 global pandemic. With a growing aging population worldwide, there is an urgent need to better understand the interactions between viruses and their aged hosts in order to improve survival outcomes. *Drosophila melanogaster* is a valuable model for the studies of both innate immunity and aging, and can be used to model aged host-virus interactions. Previously we have shown that FHV infection results in significantly higher mortality in aged flies compared to young flies regardless of sex, and likely because of impaired disease tolerance to infection. We also

demonstrated that older flies mount qualitatively distinct transcriptional responses to FHV than younger flies. Here, using 150 lines from the *Drosophila* Genetic Reference Panel (DGRP) we show that the age-dependent response to FHV infection has a genetic basis, leading us to hypothesize that variations in the genome associate with age-dependent survival of FHV. A genome-wide association study (GWAS) using these lines identified 36 *loci* respectively with a discovery p-value <1E-05, variations of which associate with age-dependent survival of FHV. Among these, 16 *loci* correspond to genes with human orthologs. Here, we are testing whether a causative relationship exists between candidate genes' expression levels and the response to virus infection with aging. Using the GAL-4/UAS system and RNAi, we are ubiquitously knocking down (KD) these candidates and comparing survival of FHV between young and aged flies and controls of both sexes. For one gene, *CG33143*, our preliminary results show that the aged KD females outlive both young KD females and controls following infection with FHV. These results were not seen in the male KD flies pointing to sex-specific effects. Currently we are measuring viral loads in experimental and control flies, with the goal to determine whether expression levels of *CG33143* impact survival outcomes via modulation of disease resistance or tolerance to FHV infection.

680F **Annotation and Phylogenetic Analysis of STUB1 and Sdr Across *Drosophila* Species** Reese Saho, Jennifer Cifranic, Annie Richters, Jamie Siders School of Science, Technology, and Mathematics, Ohio Northern University

The insulin signaling pathway is a highly conserved regulatory pathway across animal species due to its involvement in metabolism and cellular growth and development. There are over 70 different genes known to be involved in the insulin signaling pathway (ISP), notably the insulin receptor protein. However, dysfunction in any of these genes can lead to the development of diabetes, which occurs when the body fails to produce enough insulin (Type I) or insulin receptors do not properly respond to the insulin that is produced (Type II). This results in an inability to regulate blood sugar levels in the body, which can contribute to weight gain and the development of diabetic ketoacidosis. Diabetes decreases the quality of one's life at any age, making it critical to investigate all facets of how the ISP functions and how it has evolved into such a critical and robust network in animals. The Genomics Education Partnership (GEP) is specifically interested in investigating how the location of a gene within the complex architecture of the ISP influences its rate of evolution. Using a comparative genomics approach, the GEP is systematically annotating all of the genes in the insulin signaling pathway across 28 *Drosophila* species. It is hypothesized by the GEP that genes that serve as hubs, which interact with several other genes in the pathway, evolve less quickly than spokes, which interact with relatively few genes. The current work annotated 2 genes, *Sdr* and *STUB1*, across 16 species each in the *Drosophila* genus. Annotation of *Sdr* and *STUB1* using the UCSC genome and subsequent phylogenetic analysis using MUSCLE alignment suggests that Sdr may be more variable than *Sdr*, despite the fact that *Sdr* is suspected to interact with more products in the ISP than *STUB1*.

681F **Integrating multi-omics datasets across diverse species to characterize the effects of genetic variation** Heidi S Fisher[1], Gaurab Mukherjee[1], Jason A Bubier[1], Alexander Berger[1], Robyn L. Ball[1], Erich J. Baker[2], Elissa J. Chesler[1] [1]The Jackson Laboratory, [2]Belmont University

The accumulation of genomic, genetic, and epigenetic data from diverse experimental systems and model species presents significant challenges and opportunities for integrating these complex datasets. Collectively these data provide a powerful resource for researchers to both identify genomic features that are associated with phenotypic variation and to functionalize the role of genetic variants within molecular pathways and disease related networks. Here we show the integrated capabilities of two open-access web-based tools to explore, compare and contrast heterogenous genetic and genomic datasets across species. GeneWeaver is both a curated data repository and a platform to analyze and harmonize gene sets from various sources and divergent experimental systems. Recent upgrades include a transition to a cloud-based infrastructure to increase efficiency, improvements to the data model to allow batch uploads from multiple sources, including public data archives, and expanded features to reflect multiple data scores and potential sources of bias. VariantGraph is a new graph database that efficiently connects genetic variants and regulatory features to orthologous target genes across humans and model systems to enable the identification of variants in potentially non-conserved sequence that have conserved, context specific gene regulatory effects. Using streaming technology and database batch import, VariantGraph extracts large-scale relationships among datasets by parsing the data sources and building relational databases. We present the utility of these tools by exploring the genetic basis of a complex behavioral phenotype, opioid use disorder, by integrating genomic data from human populations with extensive genetic and transcriptomic data from model organisms. Together, GeneWeaver and VariantGraph allow users to efficiently connect and traverse complex genetic datasets from multiple sources, ingest new data, and interact with existing datasets for a variety of multi-omics approaches.

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682F **GenomeMUSter: A uniformly dense comprehensive mouse variation analytical resource for genomic analyses** Robyn L Ball, Hongping Liang, Molly A Bogue, Vivek M Philip, Elissa J Chesler The Jackson Laboratory

In recent years, a subset of diverse mouse strains including the Collaborative Cross, BXDs, and the classical inbred strains have undergone whole genome sequencing providing dense variant sets that can be used in genomic analyses to identify genes and genetic variants underlying complex traits and diseases. Many other strains, including some wild-derived strains have been genotyped at varying densities. However, due to the sparsity of genotyped datasets and the disparate coverage across the genome, these data are not easily combined or readily accessible for use in analytical pipelines, such as GWAS meta-analysis. We created GenomeMUSter (https://muster.jax.org), a web- and programmatically-accessible data service that includes observed and imputed allelic states for 657 strains across 106.8M segregating sites. Sixteen variant datasets were merged and harmonized. Missing allelic states were imputed using a data-driven approach that incorporates *local* phylogenetic distance. The median strain-specific imputation accuracy on a 'held-out' test set was 0.944, interquartile range [0.923, 0.991]. As a uniformly dense analytical resource, GenomeMUSter provides the genomic foundation for genetic and genomic analyses, such as identifying genes and variants related to complex disease through cross-trait cross-population GWAS meta-analyses and prioritizing mouse backgrounds for pre-clinical studies. Funding provided by NIH DA037927, DA028420 and by The Jackson Laboratory, The Cube Initiative Program Fund.

683F **Affordable short-read genome assembly allows diverse downstream genetic analyses on non-model fishes** Eric Garcia[1,2], Roy Roberts[3], Brendan Reid[4], Rene Clark[4], Jem Baldisimo[2], John Whalen[2], Abner Bucol[5], Ivan Lopez[2], Malin Pinsky[3], Kent Carpenter[2], Chris Bird[1] [1]Texas A&M University-Corpus Christi, [2]Old Dominion University, [3]University of California Santa Cruz, [4]Rutgers University, [5]Silliman University

Many genetic analyses require or are improved by utilizing a reference genome for the focal organism. However, given that producing the long-read data that is traditionally used in the assembling process remains expensive, we still lack genomic information for the great majority of non-model species. Here, we test the performance of four commonly used tools (ABySS, HipMer, SOAPdenovo, and SPAdes) in generating short-read *de novo* assemblies from eight marine fish species with contemporary and historical samples (collected over 100 ya) available. We demonstrate that assemblies with high values for N50 and BUSCO Complete and Single Copy are possible using only short-read data, and highlight a case study where this type of assemblies can later be used in combination with capture and lcWGS to investigate the loss of genetic diversity in marine fish populations from the Philippines as a result of anthropogenic impacts (overfishing and habitat degradation). Overall, successful genome assembly from short reads allows for a large suite of downstream genetic analyses from contemporary or museum specimens, and represents a new paradigm for studying non-model organisms, especially for large-scale projects, programs monitoring genetic diversity, or studies with limiting resources.

684F **Modified Mechanisms of Chromosome inheritance in the Trioecious Nematode *Auanema rhodensis*** Liesl G Strand[1], Pablo M Gonzalez de la Rosa[2], Sally Adams[3], Mark L Blaxter[2], Andre Pires da Silva[3], Anne M Villeneuve[4] [1]Developmental Biology, Stanford University, [2]Tree of Life, Wellcome Sanger Institute, [3]School of Life Sciences, University of Warwick, [4]Departments of Developmental Biology and Genetics, Stanford University

Auanema rhodensis is an unusual three-sexed nematode species in which XX females, XX hermaphrodites, and XO males co-exist in natural populations. *A. rhodensis* also exhibits unusual patterns of sex chromosome inheritance, driven in part by alterations of the oogenic meiotic program that result in the production of haplo-X oocytes in females, but nullo-X oocytes in hermaphrodites. We set out to explore the mechanisms underlying this meiotic variation by examining the cytological distribution of histone modifications associated with active and repressive chromatin, which revealed that *A. rhodensis* displays distinct patterns of germline and chromosome organization during meiotic prophase that diverge from those found in the well-studied Caenorhabditis genus. Notably, sex chromosome organization in both hermaphrodites and females appears distinct from the autosomes, with the X chromosomes being highly enriched for the repressive chromatin mark H3K9me3. Despite enrichment for this repressive mark, however, the X chromosomes appear much larger than the autosomes during diakinesis, when chromosomes condense in preparation for the meiotic divisions. This finding was unexpected based on the previously-published *A. rhodensis* short-read genome assembly, which had indicated an X chromosome half the size of autosomes. The basis of this discrepancy became clear following a new genome assembly derived from accurate long-read sequencing and Hi-C data, which revealed that roughly half of the genome is composed of extensive multimegabase blocks of repetitive DNA that are eliminated from the somatic genome during development. Using FISH probes targeted to germline-specific and somatic-retained repeat sequences, we have established that programmed DNA elimination (PDE) in somatic cells occurs subsequent to the 8-cell stage, with germline-specific repeats becoming largely restricted to apparent germline founders by the 16-cell stage and clearance of micronuclei containing eliminated DNA by the 64-cell stage. We have also used FISH to confirm the unusual patterns of meiotic sex chromosome inheritance inferred from the genetic data and to demonstrate lack of chiasmata between X chromosomes in hermaphrodite oocytes. We are intrigued by the possibility that the large repetitive chromosomal regions maintained in the germline may play structural or functional roles that contribute to the atypical segregation patterns observed during spermatocyte and oocyte meiosis.

685F **Optimal thresholds for variant impact prediction in human genes appear to depend on selection** Brynja Matthiasardottir[1,2], Stephen Mount[1], Daniel Kastner[2] [1]CBBG, University of Maryland, [2]Inflammatory Disease Section, NIH, NHGRI

Immunological disorders caused by dysregulation of the immune system include allergy, asthma, autoimmune diseases, autoinflammatory syndromes and immunological deficiency syndromes. The identification of over 450 genes underlying human immunological diseases has revolutionized our understanding of innate and adaptive immunity and provided the basis for life-saving therapies. However, the diagnostic yield of patients suffering from these disorders remains low, and many variants of uncertain significance (VUS) occur in known immune genes.

We find that validated pathogenic variants in these genes often score below standard thresholds for several tools that predict variant impact, which suggests a high false negative rate. Most methods for variant impact prediction apply the same model to all genes and assume that conservation positively correlates with functional importance. These methods have been trained using known variants in ClinVar. Variants associated with immune diseases are underrepresented in ClinVar, so it might be that existing mutation pathogenicity prediction tools are not well-suited to these genes. We find that the optimal accuracy thresholds for binary classification of variants associated with autoinflammation, found in the Infevers database, are lower than those for validated variants in ClinVar (0.324 vs. 0.65 for REVEL and 17.24 vs. 20.0 for CADD; this same trend is seen for ten other predictors).

We speculate that this difference is due to the nature of selection on these genes, with episodes of strong selection for increased inflammation due to pathogen exposure balanced by selection for reduced inflammation otherwise. In support of the hypothesis that strong but variable selection is responsible for the difference in thresholds, we verified that many genes with either extreme iHS or Fst scores are involved in immunity. Furthermore, variants in the top iHS and Fst decile of genes that are in ClinVar also show significantly lower thresholds for optimal accuracy.

Our study provides evidence that optimal variant curation guidelines may depend on the history of selection for each gene. We are exploring the relationship between measures of selection and optimal accuracy thresholds further and developing a new method of variant effect prediction that accounts for this.

686F **Pharmacogenomic Profiling of the Mu Opioid Receptor Reveals Mechanisms of Opioid Resistance in Human Patients** Ping Guo[1], Alexander Sebastian Hauser[2], Tao Che[3], Justin English[1] [1]Biochemistry, University of Utah, [2]University of Copenhagen, [3]Washington University in St. Louis

The mu opioid receptor (OPRM1) is the molecular target of opioid analgesics, first line therapies for the treatment of pain. While their therapeutic potential remains unmatched, opioids are also fueling a substance use disorder epidemic in the United States and across the world, with estimates of opiate misuse approaching 60 million individuals in 2021. However, the experience of individuals consuming opiates varies widely, from euphoria and nausea to individuals that experience no analgesia whatsoever. We hypothesized that this variability in patient response may be encoded directly in the many unstudied missense variants of OPRM1. We therefore characterized how the ten most common, but understudied, missense variants of OPRM1 (>1/10,000 individuals) altered the molecular pharmacology of these important drug targets. This study revealed multiple variants that significantly disrupt normal receptor function, including a mechanism for how one variant acts as a dominant negative receptor in heterozygote individuals, inhibiting all opioid activity in human patients. In addition, our work establishes a model for identifying and characterizing missense mutations that augment the pharmacology G-protein coupled receptors, the most commonly targeted gene family for FDA approved drugs.

687F **Evolutionary Origins and Mechanisms of Fish Antifreeze Protein in Unrelated Taxa: Insights into New Gene Birth** Nathan Rives, Vinita Lamba, Xuan Zhuang University of Arkansas

Understanding the origin and evolution of new genes with novel functions and the genetic mechanisms behind the emergence of new traits is challenging but crucial for unraveling nature's repertoire of evolutionary innovations. Fish antifreeze proteins, displaying remarkable examples of convergent evolution, offer valuable opportunities to investigate the genetic origins and evolutionary pathways of new genes. Intriguingly, nearly identical type I antifreeze proteins (AFPI) have been discovered in four distantly related fish taxa. This study tested the hypothesis of the potential sequence convergence beyond functional convergence of this new gene, and investigate different paths through which the same gene originate from different genomic resources. we investigate the genomic origin and evolutionary process of AFPI genes in three distinct fish lineages. We sequenced the complete genome of two AFPI-containing (AFPI+), each belonging to separate lineages. We also incorporated data from other AFPI+ species available in public databases and included AFPI-lacking (AFPI-) outgroup species for comparison. We isolated the genomic loci containing *AFPI* or homologous regions and annotated the *AFPI* to illustrate their gene structure.

By comparing the new gene and their homologous sequences in these genomes, we found that the *AFPI* in the three lineages exhibit dissimilar gene structure and their neighboring genomic regions do not share microsynteny, suggesting that AFPI independently evolved in each lineage. The *AFPI* in each AFPI+ lineage was linked to homologous regions in outgroup species that contain the ancestral sequence. Interestingly, the near-identical *AFPIs* originated from different precursor genes in each lineage. They arose from functionally unrelated precursor gene, and de novo evolved coding regions with novel antifreeze functions. This study provides an illustrative example of how novel functions arise and advance our understanding of the process of new gene formation.

688F **Identification of Genetic Biomarkers for Response to Proton Radiation Therapy in PCa Patients** Ayse B Cemek[1], Johnny Velasquez[2], Luisel Ricks-Santi[2] [1]New College of Florida, [2]University of Florida

Prostate cancer (PCa) is the most common cancer in men worldwide. In 2020, according to the National Cancer Institute, 3,343,976 men have PCa in the United States, with an estimated death rate of 34,700 and an estimated new case rate of 288,300 in 2023. Currently, the prostate-specific antigen (PSA) test is the cornerstone of PCa screening. In order to measure PSA, which is a protein that is generated by both healthy and cancerous cells of the prostate gland, blood samples are taken to test PSA levels. Even though the PSA test is the main measurement for PCa diagnostics, PSA is not PCa -specific but instead prostate gland-specific which results in many inaccurate diagnoses of PCa. Additionally, depending on the age of the individual, PSA levels can be elevated without the risk of PCa resulting in high false-positive rates. A positive PSA test is usually followed by a tissue biopsy to confirm the diagnosis of PCa. Given the heterogeneity of PCa, biopsy can be imprecise and may result in complications. These biopsies are used to stratify patients into PCa risk groups based on Gleason grade, stage, and prostate-specific antigen (PSA) levels and guides PCa treatment based on the risk for recurrence and metastases. In the era of precision medicine, there is a compelling need for more precise, minimally invasive methods, such as liquid biopsies, to improve the prediction of who may be at risk for recurrence or progression at diagnosis, during and following treatment. Liquid biopsies, which can detect nucleic acids in plasma or serum, hold great promise for determining the risk for recurrence and metastatic spread in cancer patients. For many cancers, it has been shown that increasing circulating cell free (ccf) DNA is associated with adverse outcomes. Therefore, the objective of this study was to determine whether ccfDNA quantity was associated with the PCa risk group. Our methods include isolating ccfDNA from serum, determining the quantity in each sample over time, and performing statistical analysis to determine its association with the risk group. Specifically, PCa patients who are eligible for the study volunteered to have their blood to be taken for Pre-Radiation Therapy, 2 weeks, 4 weeks, 8 weeks, and 12 weeks after the Radiation Therapy. Our result indicated increasing DNA quantity was not associated with PCa risk group.

689F **A fresh look at germline mutation rates at repetitive loci with AVITI sequencing and multi-generational CEPH pedigrees** Hannah C Happ[1], Tom Sasani[2], Taeho Kim[2], Deborah Neklason[2], Aaron Quinlan[2] [1]Human Genetics, University of Utah, [2]University of Utah

By sequencing the genomes of thousands of human and primate families, the genetics community understands the factors influencing the number of new mutations passed to offspring through sperm and egg. Human genome evolution is male biased, with 80% of new mutations in a typical child originating from sperm. Moreover, the primary factor influencing the rate of germline mutation accumulation in all primates is parental age: in humans, an average of 1.5 and 0.4 additional mutations arise per year of parental age in sperm and egg, respectively. While sperm progenitor cells divide throughout male adulthood, eggs are created while the future mother develops in utero; therefore the effect of paternal age was expected under a continued replication model of germline mutation, but the maternal effect was surprising. These findings led our group and others to detail the underappreciated role of DNA damage in the accumulation of germline mutation with parental age.

In 2019, we published the first large study of inter-family paternal age effects on germline mutation accumulation by sequencing the genomes of 603 individuals from 33 large, three-generational CEPH/Utah families. We found striking variability in the accumulation of paternal germline mutations in offspring over time. In 2021, we studied *de novo* structural variants (SVs) in the CEPH/Utah pedigrees, finding at least 0.160 new SV per genome.

Taken together, we now have a detailed picture of the factors that influence point and structural mutation. Ironically, however, nearly all studies have ignored the most mutable loci in the human genome: short tandem repeats and homopolymers. The reason for this is largely technological, as short-read sequencing technologies have an elevated error rate near repetitive sequence that overwhelms the signal of true mutations and precludes studies of short-tandem repeat and homopolymer mutation.

In 2023, Element Biosciences released the AVITI sequencing platform, which achieves a substantially lower error rate within and near homopolymers. Using this technology, we are sequencing the genomes of the same CEPH/Utah pedigrees to measure germline mutation rates at repetitive loci. We will present our ongoing analysis of mutation rates as a function of polymer

composition, length, and parental age and sex. Such foundational knowledge is critical for exploring the causes of genome mutation and applying this new technology to studies of repeat-associated human disease.

690F **Genome assembly of an expanding forest pest: Dendroctonus frontalis (southern pine beetle)** Megan Copeland[1], Adekola Owoyemi[1], Michelle Jonika[1], Shelby Landa[1], Jamie Alfieri[2], Terrence Sylvester[3], Zachary Hoover[1], Claudio Casola[1], Heath Blackmon[1] [1]Texas A&M University, [2]University of Texas, [3]University of Memphis

The southern pine beetle (Dendroctonus frontalis) is a type of wood-boring pest found in the United States. Since D. frontalis depends on host trees throughout its life cycle, this species is responsible for extensive ecological damage and economic loss in the timber industry. Although it has considerable damage potential, limited information is available regarding the genomics and genetics of this beetle. We produced a high-quality, chromosome-level genome assembly using long-read and Hi-C sequencing data. Our final assembly has a size of 174MB, 707 scaffolds, and a scaffold N50 of 24.829 MB. Our assembly confirms the conservation of the core stevens elements that have been identified in other polyphagan beetles. By delving deeper into the genetic makeup of D. frontalis, this assembly provides ciritical resources for research in functional and comparative genomics.

691F **A Chromosome-Scale Reference Genome to Study the Unusual Chromosome Biology and Gene Amplification in the Dark-Winged Fungus Gnat, Bradysia (Sciara) coprophila** John M. Urban[1], Susan A. Gerbi[2], Allan C Spradling[3] [1]HHMI Research Laboratories, [2]MCB, Brown University, [3]Carnegie Institution for Science, Dept of Embryology, HHMI Research Laboratories

The dark-winged fungus gnat, Bradysia (Sciara) coprophila, is a “lower Dipteran” fly with rich opportunities for studying chromosomal phenomena and genome theatrics. For example, there are “L” chromosomes limited to the germline, and across B. coprophila’s lifecycle there is a “chromosome cycle” featuring chromosome elimination and non-disjunction events. Chromosome imprinting, the ability of a cell to differentiate between maternal and paternal copies, was first discovered in this fly several decades ago. In the various chromosome elimination events across the chromosome cycle, it is the paternal copies that are targeted. In addition to the gains and losses of specific chromosomes, Bradysia coprophila larval salivary gland cell chromosomes polytenize to over 8000 copies in each nucleus, which becomes the template for further locus-specific gene amplification to produce hundreds of thousands of copies of targeted genes, presumably needed for a pre-pupal or pupal process. Whereas these gene products may well be part of the pupal case or cocoon, we are interested in determining if a subset of secreted products of gene amplification also play a role in the collective migratory Sciarid snake behavior observed in the wild.

To pursue such studies, we produced the first reference genome for Bradysia coprophila (Bcop_v1), a highly contiguous optical-map-scaffolded long-read assembly of the male somatic genome that contains four chromosomes (Urban et al, 2017 (https://repository.library.brown.edu/studio/item/bdr:733543/); Urban et al 2020 (https://doi.org/10.1101/2020.02.24.963009); Urban et al 2021 (https://doi.org/10.1186/s12864-021-07926-2)). Although this first reference assembly proved useful, the majority of contigs were not assigned to chromosomes nor were they in chromosome-scale scaffolds. Therefore, we updated it to resolve these weaknesses, producing Bcop_v2, which contained the first sequence models of each somatic chromosome (Urban et al, 2022 (https://doi.org/10.1101/2022.11.03.515061)). The chromosome-scale reference has enhanced our study of larval gene amplification. Although deep sequencing of salivary gland genomic DNA allowed us to identify amplified loci on more fragmented assemblies, the chromosome-scale scaffolds more readily allow us to infer to which previously known chromosomal locus each amplicon corresponds.

692F **PERCEPTIVE: a (Pipeline for the pRediCtion of EPigenetic modulaTors in noVel spEcies)** Eric M Small, Christina R Steadman Earth and Environmental Sciences, Los Alamos National Laboratory

ABSTRACT: Epigenetic marks, including histone post-translational modifications (PTMs) and the methylation of nucleic acids, have been shown to play key roles in regulating gene expression, genome stability, and chromatin architecture in model organisms. As novel species are identified and characterized, the role epigenetics may play in regulating their genomes and behaviors remains unexplored. However, given the extraordinary conservation of epigenetic mechanisms from budding yeast to humans, in many cases, it is possible to infer how gene expression, genome integrity, and chromatin are regulated in novel species by simply identifying epigenetic modulators or those proteins responsible for regulating epigenetic marks. Despite this, the cumbersome nature and species-dependent attributes (e.g., antibody specificity) of most wet-lab methodologies to study epigenetics preclude the rapid identification of epigenetic marks and epigenetic modulators in novel species. To address this problem, we have developed a one-click graphical software package: PERCEPTIVE (Pipeline for pRediCtion of EPigenetic modulaTors in noVel spEcies) that solely uses a species genomic sequence (plus transcriptome data if available) and preexisting information from model species to predict the epigenetic modulators and associated epigenetic marks a novel species may possess and presumably utilize. This package consists of a pipeline for genome annotation, which relies on existing software

packages and hidden Markov models to identify and annotate genes in a species of interest. These genome annotations are written out for downstream user-defined genomic experimentation and then automatically fed into a graphical application that extracts evidence for epigenetic modulators and histones. This interface provides a literature-based interpretation of results, enabling users to quickly understand potential epigenetic mechanisms in their species of interest and plan follow-up experiments. Moreover, in tandem, this pipeline extracts histone sequence information for comparison against model species, which provides invaluable information about potential histone PTMs and guides the design of sequence-specific wet lab experiments (e.g., antibody selection). Overall, this one-click software package does not require a priori knowledge of epigenetics, extensive literature review, or familiarity with command-line bioinformatics tools, making it accessible to all users who are familiar with a standard graphical computational environment.

693F **Xenbase: Improvements in *Xenopus* gene nomenclature annotations** Andrew J Bell[1], Christina James-Zorn[1], Taejoon Kwon[2], Malcolm Fisher[1], Konrad Thorner[1], Virgilio Ponferrada[1], Ngoc Ly[1], Stanley Chu[3], Sergei Agalakov[3], Bradley Arshinoff[3], Kamran Karimi[3], Troy Pells[3], Vaneet Lotay[3], Elspeth Bruford[4], Tsviya Olender[5], Peter Vize[3], Aaron Zorn[1] [1]Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, [2]Department of Biomedical Engineering, Ulsan National Institute of Science and Technology (UNIST), [3]Department of Biological Sciences, University of Calgary, [4]HUGO Gene Nomenclature Committee (HGNC), University of Cambridge, [5]Department of Molecular Genetics, Weizmann Institute of Science

Xenbase, the *Xenopus* Model Organism Knowledgebase, is the official centralized resource for gene, genome and biological research data supporting the international *Xenopus* frog research community. Xenbase continues to play a foundational role in supporting basic biomedical science, developmental, cell and cancer biology, among other disciplines, as key questions are often first explored in the *Xenopus laevis* and *Xenopus tropicalis* model organisms. Xenbase sources genome build data from the UCB *Xenopus* genome group, the NCBI and ENSEMBL, to produce curated gene page entities and integrated Xenbase general feature format (GFF) genome annotation files across successive versions. The most recent genome builds for *X. laevis* and *X. tropicalis* had thousands of instances where previously well characterized genes lost key identifying metadata such as gene symbols and gene names. This presents a major opportunity to improve the utility and potential impact of the latest *X. laevis* and *X. tropicalis* genome annotations, by addressing the underlying gene nomenclature metadata of individual genes and large gene families that lost this key information, in addition to those that have never had proper annotations. In this poster, we outline the various approaches and strategies we have undertaken at Xenbase and in collaboration with domain experts around the world to address gene symbol and gene name assignments for specific groups of genes and for large gene families. In an effort to first carry over annotations from v9 to v10 genome builds, we were able to recover annotations for hundreds of genes based on sequence similarity and by assigning gene synteny scores. We also addressed specific gene families, naming over one thousand genes belonging to the Olfactory Receptors (OR), the interleukin immune genes, Heat Shock Protein Beta (HSPB) genes and the FC receptor gene families. Meanwhile, we are in process of integrating data sharing pipelines with the Alliance of Genome Resources group in a manner that will facilitate future integration with the gold standard DIOPT pipeline for making automated gene annotations by identification of cross-species gene orthologs from protein sequences. We further collaborate directly with individuals in the *Xenopus* research community who contact us to resolve gene nomenclature issues for key genes of import to their research. Xenbase is funded by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD/NIH).

694F **Potential Role in Cell Wall Integrity of a Gene of Unknown Function in *Saccharomyces cerevisiae*** Claire E. Magill, Jill B. Keeney Biology, Juniata College

Despite the yeast *Saccharomyces cerevisiae* being an intensely studied model organism, there remain hundreds of genes of unknown function. The Yeast ORFan Gene Project is an undergraduate and faculty research project to attempt to determine the molecular functions of these genes. I am studying *FYV8* (*YGR196C*), a protein of unknown function required for survival upon exposure to K1 killer toxin. The molecular function and biological process of *FYV8* are unknown; cellular component localizes to the cytoplasm in a large-scale study. K1 killer toxin is a protein secreted by *Saccharomyces cerevisiae* that kills sensitive yeast strains. Killer yeast cells require self-immunity mechanisms against their own toxin. Past experiments confirmed the killer toxin sensitive phenotype. Bioinformatics investigations reveal *FYV8* may be involved in chitin cell wall biosynthesis. Experiments have revealed that *fyv8Δ* is sensitive to Calcofluor White, a dye that impacts cell wall stability and acts as a chitin antagonist. A related gene of interest, *ECM13* (*YBL043W*), also displays sensitivity to Calcofluor White. *ECM13* is a protein of unknown function induced by treatment with 8-methoxypsoralen and UVA irradiation and is thought to be involved in cell wall biosynthesis. Molecular function, biological process, and cellular component are unknown. Current experiments include testing the *fyv8Δ* and *ecm13Δ* on antifungal medications because these drugs impact cell wall architecture. Preliminary results suggest that *fyv8Δ* confers sensitivity to and *ecm13Δ* confers resistance to undecylenic acid and zinc undecylenate, an antifungal that is thought to interact with cell membrane components of yeast to inhibit biofilm formation or morphogenesis. Current experiments include testing *fyv8Δ* on isolated undecylenic acid to determine if there are different phenotypes

between the antifungal cream and undecylenic acid liquid with plans to test *ecm13Δ* as well. Future experiments include addressing the solubility issues of undecylenic acid by combining it with L-arginine to make it soluble in water to better perform assays. Other future experiments include testing the deletion strain for other toxic phenotypes.

695F **Specific high effect mutations in clinical and experimentally evolved *Saccharomyces 'boulardii'* isolates show that genes involved in chemical response might have a role during the adaptation to the human host** Alexandra Imre[1], Renátó Kovács[2], Ibrahim Al' Abri[1], Nathan Crook[1], Walter P. Pfliegler[3] [1]Department of Chemical and Biomolecular Engineering, North Carolina State University, [2]Department of Medical Microbiology, University of Debrecen, [3]Department of Molecular Biotechnology and Microbiology, University of Debrecen

Among dietary supplements, *Saccharomyces 'boulardii'* probiotics stand out not only with their outstanding health promoting effects, but also their ability to survive hostile niches in the human body, like the acidic environment of the stomach or the 37 °C body temperature. However, in the case of immunocompromised or severely ill hospitalized patients there is a high risk of developing fungemia. Despite several such cases are reported every year, the genetic causes of virulence are under-researched. Hence, we applied experimental evolution combined with a comparative genomics approach to identify specific mutations in 38 clinical *S. 'boulardii'* isolates and 18 isolates subjected to in vivo or in vitro experimental evolution. During the in vivo experiments the CNCM I-745 commercial isolate was injected into the bloodstream of immunosuppressed BALB/c mice, then yeast colonies were re-isolated from the kidneys followed by Illumina whole genome sequencing. The same commercial isolate was cultured in liquid Synthetic Defined (SD) media in the presence of NaCl or fluconazole, to serve as a model for stress environments yeast cells were potentially exposed to in the kidneys and in the human body, respectively. Additionally, germ-free mice were gavaged with commercial isolates from various sources. These were able to colonize the GI tract, thus isolates from fecal material were collected after 5 weeks and sequenced.
The annotation of de novo mutations showed that the clinical and evolutionarily evolved isolates have specific variants that affect protein function. These high-effect mutations resulted in numerous loss-of-heterozygosity (LOH) and gain-of-heterozygosity (GOH) events. In total 84 genes were affected by these mutations. Published large scale studies revealed that some of these genes have a role in competitive fitness, resistance to chemicals and heat sensitivity. Genes with high effect mutations were annotated to 53 GO Terms in total, among which the GO Term "response to chemical" contained the most genes after Go Term Mapping. These data indicate that the human host might exert selective pressure on the probiotic yeast isolates resulting in specific mutations that potentially have a substantial role during the adaptation of the probiotic yeast to the human host.

696F **A minimal *Saccharomyces cerevisiae* chromosome** Zachary Krieger, Cara Hull, Alessandro Coradini, Ian Ehrenreich Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California

Identifying minimal sets of genes that produce viable eukaryotic cells will reveal the core cellular components required for this domain of life. Minimization, the systematic removal of as many genes as possible from a chromosome or genome, can empirically define such minimal gene sets. However, methods for deleting large numbers of non-adjacent genes and producing chromosomes with minimal gene content are needed to make minimization possible in eukaryotes. To enable chromosome minimization in a eukaryote, we developed a method called MoSAIC (Minimal or Streamlined Architectures of Individual Chromosomes) in the budding yeast *Saccharomyces cerevisiae*. MoSAIC leverages the extensive genomics data available for this key model organism to experimentally generate and test libraries of reduced versions of an *S. cerevisiae* chromosome, some of which may have minimal gene content. To generate libraries of chromosomes that have substantially reduced gene content and give rise to viable cells, MoSAIC utilizes *in vivo* recombination between a native chromosome and synthetic DNA fragments containing known essential genes from that chromosome. Using MoSAIC, we have generated versions of *S. cerevisiae* Chromosome I from which >50% of the genes have been deleted and expect to have achieved much greater chromosome reduction by the conference. I will discuss our current work and our plans to utilize MoSAIC to generate a minimal eukaryotic genome.

697F **Effect of host genotype on the genetics of fungal persistence in mice** Yunsun Eoh, Brandon Bernardo, Matthew Dean, Ian Ehrenreich Department of Biological Sciences, USC

Fungal pathogenesis is a major source of morbidity and mortality, especially in immunocompromised individuals, infecting billions and killing millions of people every year. Previous literature has established that different mice strains have varying susceptibility to fungal infection. Identifying genetic polymorphisms that enable fungi to persist in distinct mammalian host strains can reveal mechanisms underlying host-microbe and host-pathogen interactions. Previously, we used 822 DNA-barcoded, genotyped, haploid segregants from a cross between a clinical isolate (3S) and the lab reference strain (BY) of *Saccharomyces cerevisiae* to perform linkage mapping of fungal persistence in mice. This work detected 17 loci that enable yeast to persist in different organs in C57BL/6 mice. Here, we are repeating our previous work in all eight founder strains of

the mouse Diversity Outbred (DO) population. This experiment should reveal the extent to which the same or different loci influence the organ-specific persistence of yeast in other mouse genotypes.

698F **Characterizing mechanisms of yeast persistence in mice using fine-scale genetic mapping and high resolution microscopy** Brandon Bernardo[1], Yunsun Eoh[2], Chris Ne Ville[1], Jason Junge[1], Le Trinh[1], Scott Fraser[1], Matthew Dean[1], Ian Ehrenreich[1] [1]Biological Sciences, University of Southern California, [2]University of Southern California

Identifying genetic and molecular mechanisms that enable fungal infections could enable the development of better antifungal therapies. The budding yeast Saccharomyces cerevisiae is an opportunistic pathogen. This model organism can be used to better understand fungal persistence in mammalian hosts. We previously identified 17 loci that explained most heritable variation in yeast persistence across mouse host organs using a cross of the S. cerevisiae lab reference strain (BY) and 322134S clinical isolate (3S). Half of these loci showed consistent effects across organs while the other half showed antagonistically pleiotropic effects on persistence between brain and non-brain organs. We are now trying to more deeply understand how genetic polymorphisms in yeast shape the ability of strains to persist in different parts of the mammalian body. Part of this ongoing work involves resolving the previously identified loci to causal genes using a synthetic biology strategy based on CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a method our group recently developed for cloning and assembling segments of natural chromosomes. In addition, we employ high resolution imaging of yeast strains tagged with different fluorescent proteins inside of mouse hosts. Jointly, this ongoing work will determine molecular mechanisms and spatial patterns of yeast persistence in the mammalian body.

699S **Investigating the role of H3K9 tri-methylation in regulating heat-induced transposon excision** Hannah R Wilson, Nicole Kurhanewicz, Diana E Libuda Biology, University of Oregon

The proper development of haploid gametes, such as sperm and eggs, is critical for sexually reproducing organisms to faithfully pass their genome onto the next generation. Environmental stressors, such as heat stress can negatively impact genomic integrity during gamete development, leading to genetic defects, infertility, and cancer. Chromatin modifications, including H3K9 tri-methylation (me3), protect the genome from transposon-associated damage by regulating the accessibility of heterochromatin thereby repressing the expression of transposons. In *Caenorhabditis elegans,* acute heat stress causes spermatocyte-specific heat-induced transposon mobilization and increased male infertility. Recent work indicates that heat stress perturbs H3K9me3 in a sexually dimorphic manner. My research is investigating how the chromatin modification, H3K9me3, regulates sexually dimorphic heat-induced transposon activity. To determine whether H3K9me3 is associated with heat-induced transposon excision, I am comprehensively defining the heat-induced transposon landscape across the *C. elegans* genome and comparing this dataset to the sex-specific H3K9me3 landscape. Furthermore, I am using germline histone methyltransferase mutants to determine whether H3K9Me3 differentially regulates heat-induced transposon activity in developing sperm and eggs. Lastly, I am determining whether the complete loss of histone H3K9Me3 impacts heat-induced male infertility. Overall, these studies will illuminate sexually dimorphic mechanisms that regulate transposon activity in developing gametes to maintain genomic integrity for fertility.

700S **Investigating the impact of seasonality on the gut microbiome in *Drosophila melanogaster*** Harjit Khaira, Nichole Broderick Johns Hopkins University

The interaction between the gut microbiome and its host has been studied in many species. Particularly, in the context of human health, a wide variety of host conditions and diseases has been linked to the microbiome. The gut microbiome is impacted by environmental factors such as diet, antibiotic intake, and air pollution. Diurnal changes of the environment are sensed by the master circadian clock in the superchiasmatic nucleus (SCN) region of the brain. Which controls behaviors like feeding time, and the sleep wake cycle. The gut microbiome and the host also undergo diurnal changes in composition and function that are regulated by daily feeding rhythms. This suggests that diurnal change in light conditions can impact the gut microbiome. It is known that humans experience a seasonal shift in diet which in turn can alter the microbiome composition of the gut. Seasonal changes mean changes in day-length (photoperiod) and temperature. Abrupt changes in photoperiodic conditions can have detrimental effects on human health, such as development of certain mood disorders and reproductive consequences. However, the effects of seasonal changes on the gut microbiome remains poorly understood. To address this knowledge gap, we use *Drosophila melanogaster*, which has proven to be a powerful model to study the gut microbiome due to its simple composition (5 to 20 microbial species) and ease of manipulation in the lab. Here, we report that shifts in photoperiod and temperature alter microbiome composition in *D. melanogaster*. In addition, cold temperature increases overall microbiome abundance. Shifts in photoperiod and temperature also impact host physiology, including impacts on metabolism, reproduction, and immunity. We will discuss the relative contribution of microbiome to these host phenotypes. Altogether, our study will determine how the microbiome is impacted by seasonal changes and identify its subsequent impacts on host physiology.

701S **Pinpointing the impact of a commonly used *nanos-Gal4* driver on *I-R* hybrid dysgenesis** Mia J Willingham[1], Justin Blumenstiel[1], Kelly Van Vaerenberghe[2] [1]Ecology and Evolutionary Biology, University of Kansas, [2]University of Kansas

Hybrid dysgenesis is a phenomenon of hybrid sterility that arises when paternally transmitted transposable elements absent in the mother become activated in the germline. Because transposable elements are broadly distributed through the genome, genetic analysis of hybrid dysgenesis with commonly used genetic tools is challenging. This is because moving these tools into the background of the strains of the dysgenic syndrome is likely to alter the transposable element profile, confounding clean genetic analysis. In the course of our investigations of *I-R* hybrid dysgenesis, we found that even after ten generations of backcrossing, a commonly used *nanos-Gal4* transgene altered the rate of sterility. We attributed this to two possible causes. First, the transgene might have disrupted the gene *CG6325,* thus modulating the sterility phenotype. Alternatively, the transgene might be on a haplotype that differs from the original inducer strain with respect to nearby *I* element insertions. To investigate these possibilities, we first performed strain specific CRISPR knockout of *CG6325* with non-homologous end joining. Preliminary data suggests that the impact of the *nanos-Gal4* insertion on *CG6325* function is unlikely to be the cause of altered rates of sterility. We are now performing whole-genome Oxford Nanopore sequencing of the different strains to determine if differences in sterility among crosses with different strains are more likely explained by differences in the *I* element profile of the strain carrying the *nanos-Gal4* insertion. By combining the tools of strain specific CRISPR and Oxford Nanopore sequencing, we thus illuminate a path forward for clean genetic analysis of syndromes of hybrid dysgenesis.

702S **Annotation of Thor in Drosophila albomicans: Detangling the Insulin Signaling Pathway of *Drosophila*** Laury A. Valentin Rodriguez[1], Chinmay P. Rele[2], Alondra M Diaz-Lameiro[3] [1]Biology, University of Puerto Rico-Mayaguez, [2]The University of Alabama, [3]Biology, University of Puerto Rico at Mayaguez

The insulin signaling pathway is well-conserved across animals, including humans, due to its critical role in growth and metabolic homeostasis. An evolutionary analysis of these genes in *Drosophila* can provide insight into how complex diseases related to this pathway, such as diabetes, develop in humans. As part of the Pathways Project of the Genomics Education Partnership (GEP), the *Thor* gene of the insulin signaling pathway, which contributes to translation regulation, response to environmental stress, and cell growth regulation, was annotated in *D. albomicans* based on conservation of local synteny and parsimony with *D. melanogaster*. After identifying the genomic location of the putative ortholog through a protein alignment of *Thor* in *D. melanogaster* against a scaffold of *D. albomicans* using NCBI BLAST, the genomic neighborhood of this ortholog was examined using synteny to the *Thor* neighborhood in *D. melanogaster*. Based on local synteny analysis, *Thor*'s gene structure in *D. melanogaster* and the alignment to *D. melanogaster* proteins, gene prediction models, RNA-Seq data, and splice junction sites in the UCSC Genome Browser, the two coding exons of the putative ortholog were localized and annotated. Although the genomic neighborhood of the putative ortholog did not share synteny with *Thor*'s neighborhood in *D. melanogaster*, a comparative analysis of *Thor*'s genomic neighborhood across different *Drosophila* species between *D. melanogaster* and *D. albomicans* revealed that this neighborhood has gone through recombination during the speciation of the genus. This analysis and the annotation supported a putative ortholog of *Thor* in *D. albomicans* and revealed a *Thor* gene duplicate, which has been observed in other basal *Drosophila*. This duplicate shares the same gene structure and coding exon as the ortholog and only diverges from it by a few amino acid changes. *Thor's* conservation, regardless of changes in local synteny, suggests an essential role in the insulin signaling pathway. Future gene annotations of this pathway across different *Drosophila* species through GEP could provide a better understanding of its evolution and function and the role of genes such as *Thor* in diseases such as diabetes.

703S **Adaptive TE insertions in a long non-coding RNA: a novel class of transcriptional repressors?** Erin K Kelleher[1], Savana Hadjipanteli[2], Natalie Copeland[1] [1]Biology and Biochemistry, University of Houston, [2]Cell and Molecular Biology, University of Pennsylvania School of Medicine

Transposable elements are genetic parasites whose mobilization throughout the genome is a major source of deleterious mutations. However, some TE insertions are beneficial and are positively selected because they improve host fitness. Adaptive TE insertions have been shown to alter the function of adjacent genes by positively and negatively impacting their expression. Alternatively, individual TE insertions can also be adaptive because they establish repression of transposition through piRNA production or other mechanisms. In a recent laboratory evolution experiment, we discovered that *lncRNA43651* is an adaptive insertion hot spot for *P*-element DNA transposons in *Drosophila melanogaster*. However, the functional effects of these insertions on adjacent gene expression or *P*-element repression remain unknown. To this end, we examined the effects of *lncRNA43651* insertions on viability, *P*-element transcriptional regulation and piRNA biogenesis. We determined that although *lncRNA43651* insertions exhibit recessive viability effects, these fitness costs may be offset by dominant beneficial effects on *P*-element regulation. *P*-element insertions in *lncRNA43651* exhibit stronger transcriptional repression of P-element promoters than known repressive insertions in traditional piRNA clusters. Furthermore, the presence of *lncRNA43651* insertions enhances P-element piRNA production initiated from cluster insertions. However,

the *lncRNA43651* insertions themselves are not a significant source of ovarian *P*-element derived piRNAs. Our findings point to a new class of repressive TE insertions, that establish and enhance piRNA-mediated silencing through an unknown mechanism.

704S **New biological insights of the 3D nuclear architecture using WaveTAD, a probabilistic, resolution-free, and hierarchical TAD caller** Ryan Pellow[1], Josep Comeron[2,3] [1]University of Iowa, [2]Department of Biology, University of Iowa, [3]Interdisciplinary Program in Genetics, University of Iowa

Eukaryotic genomes fold their chromosomes within nuclei in three-dimensional space, with systematic and coordinated multiscale structures including loops, topologically associating domains (TADs) and higher-order chromosome territories. This 3D organization plays essential roles in gene regulation and development, responses to physiological stress and disease. Our understanding of the 3D organization of genomes has benefited from the current ease to obtain sequencing data that identifies genomic contacts in 3D space. These genomic datasets are, however, highly sparse and noisy and current methodologies to infer 3D structures show limitations and biases, including i) different outcomes depending on the resolution of the analysis and sequencing depth, ii) results that are only qualitative and prevent statistical comparisons, iii) no insight into the frequency of the structures that can exist in a sample with multiple genomes, and iv) no direct prediction of hierarchical structures. These caveats hinder rigorous and objective comparisons of 3D properties across genomes and between experimental conditions or species.

To address all these challenges, we developed a wavelet transform-based method (WaveTAD) that describes the 3D nuclear organization in a resolution-free, probabilistic, and hierarchical manner. Besides being the only resolution-independent method, WaveTAD shows increased accuracy and sensitivity than current approaches, predicts multi-layered structures and generates probabilities associated with each structure that capture variable frequency within samples. We applied WaveTAD to multiple datasets from *Drosophila*, mouse, and humans to illustrate new biological insights that our new method provides, including extensive embryonic 3D organization before zygotic genome activation, the quantitative effect of multiple CTCF units on the stability of loops and TADs, the association between histone marks and transcription factors with TAD structures and strengths, or the analysis of COVID patients unveiling 3D genomic changes not previously identified.

705S **The role of *Lactiplantibacillus plantarum* in the gut microbiota-brain axis - implications for aging** Melanie Reinoso, Caroline Casiano, Charles Pfeiffer, Josue Rodriguez-Cordero, Alfredo Ghezzi, Jose Agosto, Imilce Rodriguez-Fernandez University of Puerto Rico Rio Piedras

The gut microbiota-brain axis is a bidirectional communication between the resident microbes, the gut, and the brain; it plays a pivotal role in aging and age-related diseases, particularly neurodegenerative conditions. Probiotics have emerged as a promising avenue for interventions targeting age-related diseases by modulating this axis. In this study, we explore the effects of the commensal bacteria and probiotic *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) on the gut-brain axis in the fruit fly *Drosophila melanogaster* by using an untargeted metabolomics approach.

Flies are a genetically amenable model, display age-related phenotypes, and have a simpler microbiota than mammals that is easy to manipulate. These traits allow discoveries made in flies translatable to mammals. To understand how *L. plantarum* can provide a benefit to the health of animals we supplemented young and old flies with or without *L. plantarum*. These flies were flash-frozen and separated by heads (brain) and bodies (intestine and other organs) and collected separately. This approach allowed us to identify changes in metabolites that are age-, tissue- and treatment-specific. Our results show a total of 855 biochemicals: 798 known biochemicals and 57 unknown biochemicals. Our findings revealed substantial metabolomic disparities linked to age and body section, emphasizing the dynamic nature of the gut-brain axis in the context of aging. Notably, the influence of *L. plantarum* supplementation on the metabolome appeared relatively modest. However, there were significant differences in neurotransmitters of interest, specifically acetylcholine which is increased in the bodies of old flies treated with *L. plantarum*. Preliminary data from our lab shows that *L. plantarum* can alter sleep latency and locomotion. Our ongoing work aims to characterize the link between *L. plantarum*-mediated changes in neurotransmitters and distinct behaviors. Data obtained from this project will help us characterize the molecular mechanisms used by *L. plantarum* to influence the gut-brain axis.

706S **The impact of Piwi overexpression on *I-R* hybrid dysgenesis** Sean A Nash[1], Kelley Van Vaerenberghe[2], Justin P Blumenstiel[1] [1]Ecology and Evolutionary Biology, University of Kansas, [2]University of Montana

Drosophila Piwi is the founding member of the PIWI clade of Argonaute proteins and plays a critical role in transposon silencing in the germline nucleus via small RNAs known as PIWI-interacting RNAs (piRNAs). These piRNAs derive from fragmented transposons residing in genomic compartments known as piRNA clusters. Piwi has diverged from an ancestral gene owing to a duplication of a Piwi/Aubergine ancestor. In contrast to the nuclear function of Piwi, Aubergine is expressed

in the cytoplasm. The role that Piwi has in the establishment of piRNA biogenesis from germline dual-strand clusters is not well understood, but studies indicate that maternally provisioned piRNAs, loaded into Piwi protein in the early embryo, play a critical role in licensing their establishment. Interestingly, later in development, Piwi becomes dispensable, when the Rhino-Deadlock-Cutoff complex maintains cluster identity independent of Piwi. To investigate the role that ongoing Piwi expression has on piRNA function, we performed an analysis of Piwi overexpression in the *I-R* syndrome of hybrid dysgenesis. In the *I-R* syndrome of hybrid dysgenesis, young dysgenic females that have inherited active *I-elements* solely from their father experience a loss of fertility when their eggs fail to hatch. This is caused by lack of maternally transmitted *I-element* piRNAs and activation of the *I-element* during oogenesis. Interestingly, as they age, females become fertile again and eggs begin to hatch. This is presumably due to the slow accumulation of piRNAs that eventually restore *I-element* silencing. Our study seeks to determine the influence that Piwi overexpression has on this process and evaluate the history of Piwi gene duplication in this context.

707S **Starting to Remember: Initiation of Polycomb target gene regulation in the Early Drosophila Embryo** Sean Johnsen, Dan McKay Genetics, University of North Carolina at Chapel Hill

The Polycomb Group proteins serve in one of the most highly conserved epigenetic regulatory systems across eukaryotic life, enabling the maintenance of cell identity across mitosis through stable repression of target genes. Meiosis, however, enables a total reset of the developmentally-restricting epigenetic information, resulting in a totipotent zygotic chromatin state. From this totipotent state, developmental gene regulatory programs re-impart Polycomb-mediated gene repression in a cell-type specific manner. The onset of Polycomb chromatin domain formation coincides with the transcriptional activation of the zygotic genome. Prior to this stage in embryogenesis, Polycomb gene products are produced during oogenesis and deposited into the zygote by the mother. The requirement of Polycomb proteins during oogenesis has made genetic dissection of their role in early embryogenesis technically challenging. As a result, our understanding of de novo cell-type specific initiation of Polycomb-mediated repression remains incompletely understood. We are using recently developed conditional protein inactivation systems to determine which epigenetic information is required for proper initiation of epigenetic memory by the Polycomb regulatory system.

708S **The Relationship Between Thiamine and Drosophila Melanogaster Preference for Dietary Yeast** Dean Peterson Life Sciences, Brigham Young University

This study will focus on the microbiota's effects on *Drosophila Melanogaster* dietary preference for yeast (DPY). A study completed in our lab suggested that bacterial thiamine biosynthesis/metabolism might influence fly DPY which shifted from yeast restricted diet to yeast rich (Call 2022). I previously confirmed the prediction that thiamine influences fly DPY by showing that flies reared on thiamine supplemented diets do not display reduced DPY. I will now perform follow-up tests to determine the specificity of thiamine as the molecule causing shifts in DPY. I will test flies given other vitamin B supplemented diets. If thiamine is the only B vitamin that influences fly DPY, then these tests show that the effect is relatively specific to thiamine when compared to other B vitamins. I will also perform a parallel experiment to confirm the role of bacterial thiamine on DPY. I will rear flies colonized with bacterial thiamine biosynthesis/metabolism mutants on thiamine supplemented diets then measure DPY. If flies raised with increased thiamine in their diet prefer yeast restricted diet, then thiamine is the molecule involved in yeast preference for these mutants. Together, these approaches will reveal that thiamine is the molecule used by the microbiota to influence fly DPY.

709S **Diet reveals tissue-specific gene expression of an uncharacterized gene in *Drosophila melanogaster*** Meri Nehlsen[1], Maria Langegger[1], Julien Ayroles[2,3], David Stern[4], Luisa F. Pallares[1] [1]Friedrich Miescher Laboratory, Max Planck Society, [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [3]Ecology and Evolutionary Biology Department, Princeton University, [4]Janelia Research Campus of the Howard Hughes Medical Institute

Understanding how organisms respond to changing environmental conditions is a key goal in evolutionary biology. One of the potential mechanisms underlying such responses are Genotype-by-Environment (GxE) interactions, which describe that a genotype can produce different phenotypes depending on the environmental context. In a study of diet-dependent differential gene expression in *Drosophila melanogaster*, among many modest changes under a High-Sugar (HS) diet, we previously identified a gene with striking tissue-specific upregulation: while only moderately upregulated in the body, expression in fly heads increased 16-fold on HS diet, resulting in the strongest response to a diet high on sugar. Interestingly, this gene and its function have not been previously characterized. Here we explore the cryptic gene expression unveiled by HS diet and potential diet-dependent function of this gene.

First, to determine the protein localization, we created a fly line using CRISPR-Cas9 in which the candidate gene is tagged with hemagglutinin (HA). Using anti-HA antibodies, we detected protein expression in the gut as well as in several brain

compartments, likely glia cells, tracheas, and the mushroom body, and confirmed that the expression of the candidate gene is indeed stronger under HS diet.

Using a whole-genome gene expression dataset, we estimated the correlation between the candidate gene and every other gene in the genome for head and body, in both conditions, control and HS diet. GO enrichment analysis of the top correlated genes suggested involvement of the gene in digestive functions in the body regardless of the diet. This result aligns with the predicted chymotrypsin-like serine protease activity of this gene. In the head, under control diet the gene had no strong correlation with any gene, whereas under HS diet strong correlations with genes involved in lipid catabolism were unveiled. This correlation suggests that the candidate gene becomes part of well-known regulatory networks in a diet-dependent and tissue-specific manner.

In conclusion, locating the expression of the gene to specific structures in the fly head, as well as determining the putative function of the gene based on co-expression with other genes provides the first step towards characterizing the molecular function of the candidate gene as well as its potential role in regulating the response to HS diets.

710S **The role of Polycomb group proteins in animal activity** rachel e ferris[1], Nicole C Riddle[2] [1]biology, university of alabama at brimingham, [2]Biology, university of alabama at brimingham

The Polycomb Group (PcG) proteins were defined initially based on their regulation of the Hox gene cluster and the homeotic mutations that result in their absence. Molecular characterization of the PcG proteins show that they modify histones and repress developmentally regulated genes, including the Hox genes, through the action of two complexes, PRC 1 and PRC 2. Our previous work has linked components of PRC1 and PRC2 to the control of physical activity, when PcG genes were identified as candidate genes in a genome-wide association study for animal activity. Here, we follow up on this initial finding and systematically remove individual components of PRC1 and PRC2 in the central nervous system to identify which PcG protein are involved in the control of animal activity levels. To achieve tissue-specific knockdown, we used the UAS/GAL4 system. While we do not see many changes in activity early in life, we find that late in life, activity levels are significantly increased in the knockdown compared to the control genotypes. Our findings further support the potential role of the PRC complexes in the control of animal activity. Identifying the major players involved in the control of physical activity will aid in the development of personalized healthcare interventions.

711S **Cas9 electroporation and AAV templates efficiently generate knock-in and conditional alleles in mice** Lauri G Lintott[1], Valerie Laurin[1], Linda Chan[1], Qing Fan-Lan[1], Marina Gertsenstein[2], Lauryl MJ Nutter[2,3] [1]The Hospital for Sick Children, [2]The Centre for Phenogenomics, [3]Genetics & Genome Biology, The Hospital for Sick Children

Introduction: Genome editing with Cas9 has simplified generation of genetically modified mice. However, targeted insertion of large fragments and one-step generation of loxP-flanked conditional alleles remain challenging. Co-incubation of recombinant adeno-associated virus (rAAV) with rodent embryos was used to deliver repair templates for Cas9-mediated genome editing to produce knock-in alleles.

Aim: Our aim was to adapt these protocols to establish a workflow to efficiently generate knock-in and conditional alleles.

Strategy: We used rAAV to deliver repair templates for conditional and knock-in alleles and Cas9 RNP to introduce DNA double-strand breaks at the target sites.

Results: We successfully generated founders with either knock-in or loxP-flanked conditional alleles. Using template-specific PCR and template copy number assessment we found that multicopy rAAV integration occurred. We also developed a founder screening pipeline to eliminate the most mice at early steps, minimizing the number of animals screened. Using founders that passed our founder quality control for germline transmission test breeding we established mouse lines with the desired allele in each case tested.

Conclusions: Template delivery by rAAV co-incubation and Cas9 RNP electroporation is a robust protocol to generate knock-in and conditional alleles.

712S **Adding the missing tiles to the puzzle: reconstruction of KRAB-ZFP gene clusters sequence and evolution in mice** Melania Bruno, Apratim Mitra, Sharaf Maisha, Ryan Dale, Todd Macfarlan The Eunice Kennedy Shriver National Institute of Child Health and Human Development, The National Institutes of Health

KRAB-zinc finger protein (KZFP) genes are an abundant and fast evolving family of transcription factors, numbering in the hundreds in most mammalian genomes.

KZFPs are characterized by the presence of a KRAB domain, often recruiting heterochromatin forming factors, and of an array of tandem C2H2 zinc fingers, conferring DNA binding specificity. Although some conserved KZFPs play a role in fundamental developmental processes, many evolutionary young and clade- or species-specific KZFPs repress the expression of endogenous retroviruses (ERVs) and, intriguingly, share a similar evolutionary age with the elements they repress. This suggests that new KZFP genes "emerge" in response to waves of ERV invasion into the host genome.

However, it is currently unknown how new KZFP genes emerge and evolve.

The mouse germ line has been recently colonized by numerous distinct retroviruses that have established ERV families unique to mice. The KZFP gene family has also recently expanded in the mouse lineage, with parallel evolution in different inbred mouse strains. This makes the mouse an ideal model to study the evolution of KZFP genes.

KZFP genes are organized in highly repetitive and dynamic genomic clusters. In the mouse, several KZFP gene clusters display evidence of recent segmental duplications, but sequence gaps in the available genome assemblies have prevented a thorough reconstruction of their evolution. We have generated de novo assemblies and filled the gaps in all KZFP gene clusters in the reference C57BL/6J and the 129S1/SvImJ mouse strains, by combining PacBio HiFi and Nanopore long read sequencing. We found that some KZFP gene clusters are much larger and repetitive than previously thought, harboring many more KZFP genes. Complete reconstruction and analysis of clusters that emerged in the Muridae clade revealed that new KZFP genes emerge by duplication of large regions harboring multiple KZFP genes. We found that one region in particular expanded from ~1Mb in rat to ~5Mb in C57BL/6J and ~7Mb in 129S1/SvImJ mice. Furthermore, comparison between C57BL/6J and 129S1/SvImJ KZFP gene clusters, revealed different modes of cluster expansion: C57BL/6J clusters display a higher degree of intra-cluster homology while 129S1/SvImJ clusters present more fragmented duplications with less overall intra-cluster homology. This suggests likely differences in the pathways that regulate the maintenance and evolution of these repetitive loci in different mouse strains.

713S **Time-series transcriptomic analysis reveals selfish B chromosome, PSR, may regulate wasp development through testis-biased gene expression** Xinmi Zhang[1], Ying Zhang[2], Xu Wang[2], Patrick Ferree[3] [1]W.M. Keck Science Center, [2]Auburn University, [3]W. M. Keck Science Center

Paternal Sex Ratio (PSR) is a selfish B chromosome found in the parasitoid wasp *Nasonia vitripennis*. PSR is transmitted through sperm to progeny and can cause Paternal Genome Elimination (PGE) in fertilized diploid embryos, leading to the conversion of diploid female wasps into haploid males. This action leads to super-Mendelian transmission of PSR. Understanding how PSR causes PGE is important for comprehending how B chromosomes can induce genome conflict, how B chromosomes evolve, and their potential applications in insect pest control. A recent study has revealed that the PSR-expressed *haplodizer* gene is responsible for the PGE event, but it remains unclear when and mechanistically how *haplodizer* functions in this process.

To address this knowledge gap, we are performing transcriptome profiling across different developmental stages of both wildtype (WT) and PSR-carrying male wasps, from early embryos to adults. Tissue-specific and stage-specific gene expression patterns were analyzed to investigate the expression level changes of *haplodizer* and other PSR-expressed genes. Differential gene expression analysis was also conducted between WT and PSR-carrying wasps at different stages to uncover transcriptional changes caused by PSR.

Preliminary analyses of the mid pupal stage showed that out of 28 PSR expressed genes, 18 genes had low to very low expression levels (RPKM < 2). Of the higher expressed genes, 2 were testis-specific, and 7 others, including *haplodizer*, were testis-biased. Furthermore, among ca. 12,000 normal genes in the wasp's genome, 69 showed significant up-regulation in the testis and 9 genes were up-regulated in the carcass of PSR-carrying males compared to WT males. No genes were down-regulated in the testis, while 44 genes exhibited down-regulation in the carcass of PSR-carrying males compared to WT males. These preliminary findings suggested that there is a testis-specific bias for PSR-expressed genes, which may be relevant to the ability of PSR to induce PGE and favor its vertical transmission. This study represents one of the most comprehensive transcriptome-level gene expression studies for a B chromosome across development.

714S **Differential adenine methylation analysis reveals 6mA variability after experimental evolution.** Carl Stone, Megan Behringer Vanderbilt University

Methylated DNA adenines (6mA) are a critical epigenetic modification in bacteria that affect cell processes like replication, stress response, and pathogenesis. While much work has been done characterizing the influence of 6mA on specific loci, very few studies have examined the dynamics of 6mA over evolutionary time. We used third-generation sequencing technology and a newly developed differential methylation analysis pipeline to examine 6mA methylation across the Escherichia coli K-12 substr. MG1655 genome. 6mA levels were consistently high across GATC sites; however, we detected regions of decreased

6mA in the WT genome and in experimentally evolved populations from WT and methyl-directed mismatch repair-deficient (MMR-) lineages. We show that characterization of 6mA in bacterial populations is complementary to genetic sequencing and informative for molecular evolution.

715S **Genome assembly of three rhabditid species** Juan Pablo Aguilar Cabezas, Janna Fierst Biological Sciences, Florida International University

The evolution and phylogeny of nematodes has conflicts, but with the revolution in DNA sequencing sequencing, the future is very promising, potentially not only resolving taxonomic uncertainties but also shedding light on the diversity of this group. The suborder Rhabditina includes the most famous species, *Caenorhabditis elegans*, a model organisms for genetic studies. The goal of this study was to shed light into the evolution and natural history of this order by reconstructing the genome of three species at the root of the tree: Pelodera teres, Poikilolaimus oxycercus, and Bunonema sp. These data will serve to not only build a robust phylogeny of rhabditids and outgroups, but also to better estimate genome evolution such as gene gain/loss, transposable elements, and potentially new epigenetic marks.

716S **Achieving epigenetic precision: The complete human diploid reference genome of RPE-1 identifies the phased epigenetic landscapes from multi-omics data** Emilia Volpe, Luca Corda, Simona Giunta Dpt. Biology & Biotechnology Charles Darwin, University of Rome Sapienza

Comparative analysis of recent human genome assemblies highlights profound sequence divergence that peaks within polymorphic loci such as centromeres. This raises the question about the adequacy of relying on human reference genomes to accurately analyze sequencing data derived from experimental cell lines. Here we propose a novel approach, referred to as 'isogenomic reference', that leverages a matched reference genome to perform multi-omics analyses. We have generated a new diploid genome assembly for the human retinal epithelial cells (RPE-1), a widely used non-cancer laboratory cell line with a stable diploid karyotype, that presents phased haplotypes and chromosome-level scaffolds completely spanning centromeres. Using this assembly, we have characterized haplotype-resolved genomic variation unique to RPE-1, including a stable marker chromosome X with a 73.18 Mb segmental duplication of chromosome 10 translocated onto the microdeleted telomere t(X*q*;10*q*), specific to this cell line. Comparative analyses revealed sequence polymorphism within centromeric regions, including unexpected genetic and epigenetic diversity among haplotypes for all chromosomes. Using our assembly as reference, we re-analyzed both our own and publicly available sequencing, methylation and epigenetic data generated in RPE-1 which had previously been analyzed with non-matched and non-diploid reference genomes. Our results show that the isogenomic reference improves alignments with an increased mapping quality up to 85% while halving mismatches, resulting in significant changes in peaks calling related to centromeres. Notably, we were able to call the precise kinetochore site for each chromosome and show how differences between the two haplotypes, with implications in chromosome segregation dynamics. Our work represents a proof-of-concept, showcasing the use of matched reference genomes for multiomics analyses and, at scale, serves as the foundation for a call to comprehensively assemble experimentally relevant cell lines for a widespread application of isogenomic reference genomes.

717S **Next-generation map of constrained coding regions from hundreds of thousands of humans** Suchita Lulla, Aaron Quinlan, Peter McHale University of Utah

A longstanding interest in human genetics has been to identify the subset of the human genome that is critical to normal human development. In pursuit of this question, we previously created a map of constrained coding regions (CCRs) [1], or regions where the absence of genetic variation implies negative selection due to critical function during development, and in which variation is expected to cause disease. We defined a region as the coding distance (in coding space) between consecutive protein-changing variants, and we weighted region lengths by sequencing coverage. We then quantified the degree of constraint of each region in proportion to its length, where regions with the greatest distance between protein-changing variants have the highest predicted constraint. This map provided a more fine-scale metric of intraspecies constraint than other previously established gene-wide measures and nominated new protein-coding regions that are essential to human development.

We will present progress on a more rigorous approach to modeling constraint in coding space that calculates a ratio of observed to expected constraint in small sliding windows across protein-coding exons. This approach allows us to relax the original requirement of the complete absence of protein-changing variants in a CCR to capture cases where genetic variation in a region is present, but extremely sparse, which will occur more often as cohort sizes increase. The gnomAD v4 dataset provides unprecedented deep sampling of human coding variation, coupled with our more complete model of constraint, allowing us to construct a higher-resolution and more accurate map of constraint and assess which CCRs from the previous dataset survive given the expanded dataset. We anticipate that our efforts will yield a much higher resolution map of

constrained coding regions in the human genome, providing a valuable resource for rare disease interpretation and identifying coding sequence that is essential to human development.

References

1. Havrilla JM, Pedersen BS, Layer RM, Quinlan AR. A map of constrained coding regions in the human genome. Nat Genet. 2019;51: 88–95.

718S **Establishing baseline transcriptome profiling of ATCC's human and mouse cell lines** Ajeet P Singh, Rula Khairi, Amy L Reese, Jade Kirkland, Corina Tabron, Noah Wax, James Duncan, Robert Marlow, Steve King, Ana Fernandes, John Bagnoli, Briana Benton, Jonathan L Jacobs ATCC

Cell lines are essential tools in biomedical research, serving as primary resources for numerous laboratory experiments. However, cell line contamination and inaccuracies in public datasets can adversely affect research. To address the need for authenticated cell lines with comprehensive data provenance, ATCC® collaborates with QIAGEN® to establish the ATCC® Cell Line Land. This continually growing database contains baseline whole transcriptome (RNAseq) data traceable to the highly utilized human and mouse cell lines in ATCC®'s repository. By providing this data, we aim to refine cell line selection, shifting from traditional methods to decisions informed by genotype-phenotype correlations. Such informed decisions can foster reproducible research, which can reduce the timelines in the development of impactful therapeutics. Currently, we are creating over 1,000 new RNAseq data sets annually and, ultimately, sequence all the cell lines held within ATCC's collection. To date, we've sequenced over 300 cell lines from kidney, blood, and lung tissues (Over 1,500 datasets), encompassing primary, engineered, cancerous, and healthy cells. Our overarching objective is to establish research standards by offering traceable, standardized, and authenticated transcriptomic data.

In this study, we conducted a comparative analysis of the naive HEK-293 (ATCC® CRL-1573™) cell line and its derivatives, which are extensively used in virology and biomanufacturing production, and in research and development. Through transcriptome analysis, we identified molecules exhibiting both general and specific profiles across these cell lines. Our pilot study on differential gene expression specifically highlighted distinct genes and pathways in the HEK-293.2sus (ATCC® CRL-1573.3™) cells cultivated in suspension as compared to the adherent parental HEK-293 cells (ATCC® CRL-1573™). Further analysis using Ingenuity Pathway Analysis(r) unveiled dysregulated pathways and pivotal upstream regulators that influence cellular phenotypes and gene expression shifts. Recognizing the significance of HEK-293 cells in these applications, our study leverages RNAseq to shed light on unique gene expression patterns and pathways within this cell line panel, particularly under suspension conditions, offering valuable insights to optimize their use and improve therapeutic outcomes. The meticulously curated RNAseq data housed in the ATCC Cell Land empowers researchers with the ultimate decision-making tool for selecting the right cell line model for their own use cases.

719S **Diversity of cow transposable elements and developmental expression in early embryos** Guangsheng Li, Jingyue (Ellie) Duan Animal Science, Cornell University

Transposable elements (TEs) are genetic elements that replicate and mobilize within host genomes, influencing gene regulation and genome stability. However, their role in cattle genome remains poorly understood. This study aims to investigate TE diversities in cattle genome and determine their transcription during early embryo development. First, we identified that TEs constitute 46.29% of the bovine genome, with DNA transposons accounting for 2.15% and retroelements at 44.1%. To estimate TE age, we constructed phylogenetic trees for all TE families with at least 10 copies, using defragmented insertions of at least 100 bp in length (n=677), revealing a moderate correlation between TE age and TE copy numbers. Interestingly, TE length and age showed no correlation. For the genomic distribution of TEs, we observed that TEs were more enriched in intergenic and intronic regions than gene bodies, including exons, and promoters. Moreover, different TE classes exhibited correlations on their distribution, with DNA and SINEs negatively correlated with LINEs and LTRs, but positively correlated with gene distribution. Next, we analyzed 163 samples representing diverse developmental stages and embryo types, including in vivo, *in vitro,* and somatic cell nuclear transfer (SCNT) embryos at each pre-implantation stage. At the transcriptome level, TE expression clusters showed distinct changes between 4-cell and 8-cell stages in *in vivo* embryos, while similar patterns were observed between 8-cell and 16-cell stages in both in vitro and SCNT embryos. These results suggest different embryo productions impact the timing of zygotic genome activation. Overall, this study provides comprehensive profiles of TEs in the bovine genome and novel insights into the complex molecular mechanisms of early embryogenesis. These findings open avenues for further research into the functional roles of TEs in embryonic development and their implications in reproductive technology and embryo quality.

720S **The first two chromosome-scale genome assemblies of American hazelnut enable comparative genomic analysis of the genus *Corylus*** Scott Brainard[1], Tomas Bruna[2], Julie C Dawson[1], Dean Sanders[3] [1]Plant and Agroecosystem Sciences,

University of Wisconsin-Madison, [2]U.S. Department of Energy Joint Genome Institute, Lawrence Berkeley National Lab, [3]Bioinformatics Resource Center, University of Wisconsin-Madison

The native shrub *Corylus americana* is currently utilized in breeding programs developing commercially viable hazelnut varieties for the Upper Midwestern U.S. Modern breeding methods (e.g, marker-assisted selection and genomic prediction) are greatly aided by a high-quality reference genome assembly, however this is currently lacking for *C. americana*. We have therefore developed the first chromosome-scale reference assemblies for *C. americana*, using the accessions 'Rush' and 'Winkler'.

Genomes were assembled using HiFi PacBio reads and Arima Hi-C data, and Oxford Nanopore reads and a high-density genetic map were used to perform error correction. N50 scores are 31.9 Mb and 35.3 Mb, with 90.2% and 97.1% of the total genome assembled into the 11 pseudomolecules, for 'Rush' and 'Winkler', respectively. Gene prediction was performed using custom RNAseq libraries and protein homology data. 'Rush' has a BUSCO score of 99.0 for its assembly and 99.0 for its annotation, while 'Winkler' had corresponding scores of 96.9 and 96.5, indicating high-quality assemblies. These two independent assemblies enable unbiased assessment of structural variation within *C. americana*, as well as patterns of syntenic relationships across the *Corylus* genus. Furthermore, we identified high-density SNP marker sets from genotyping-by-sequencing data using 1,343 *C. americana*, *C. avellana*, and *C. americana* x *C. avellana* hybrids, in order to assess population structure in natural and breeding populations. Finally, the transcriptomes of these assemblies, as well as several other recently published *Corylus* genomes, were utilized to perform phylogenetic analysis of sporophytic self-incompatibility (SSI) in hazelnut, providing evidence of unique molecular pathways governing self-incompatibility in *Corylus*.

These assemblies are an important first step in providing a resource for using next-generation sequencing data in the improvement of *C. americana*, while also enabling comparisons of structural variation across *Corylus* species. We hope these assemblies will aide in the application of modern breeding methods to the development of commercially viable hazelnut varieties for the Upper Midwest.

721S **Qploidy: ploidy and aneuploid determination for polyploid species** Cristiane H Taniguti[1], Jeekin Lau[2], David Byrne[2], Oscar Riera-Lizarazu[2] [1]Horticultural Sciences, Texas A&M University, [2]Horticulture Sciences, Texas A&M University

In the field of plant genetics, ploidy detection has traditionally relied on methods such as microscopy, flow cytometry, or their correlation with pollen diameter. However, these methods come with a set of limitations including low precision and unreliability for many samples. In human cancer genetics studies, single nucleotide polymorphism (SNP) array technologies have been successfully used to determine ploidy and identify aneuploidy. The application of these technologies for ploidy and aneuploidy estimations in the field of polyploid plant genetics remains relatively uncommon despite the substantial volume of array data generated for other research goals in plants. The challenge in this context is the normalization of allele intensities in polyploid plant array data due to the quality and stability differences between plant and human arrays. Furthermore, the fact that available normalization methods consider predominately diploid genotype calls, a common occurrence in humans but not in polyploid plants. Thus, our study aims to develop methods that use genotypic data for these ends. First, we developed a standardization method for array data intensities to account for higher ploidy genotype calls, specifically tailored to the needs of polyploid plants. We implemented the method in an R package and Shiny app called Qploidy. To evaluate the effectiveness of this approach, we conducted experiments using a total of 1,536 rose samples from collections and mapping populations. The method successfully differentiated 176 diploid, 76 triploid, and 420 tetraploid plants, as well as seven tetraploid mapping populations (N=769). It also detected an average of 2.8% of aneuploid individuals among the tetraploids in the collections and a substantially greater proportion within mapping populations, averaging 17.4%. We explored the consequences of those aneuploids when constructing linkage maps for mapping populations. The presence of aneuploidy led to a higher number of erroneously estimated recombination breaks for the affected chromosomes since data for these did not align with the linkage map-building software's assumptions. By quantifying the number of estimated recombination breaks, we could also discern which parent of the mapping population was the source of an aneuploid gamete. The male parent was shown to be a less frequent source than the female parent (84%), revealing a stronger negative effect of aneuploidy on male gametophytes. Among tetraploids, we also observed a higher number of aneuploids with an extra chromosome (pentasomy) than with a reduced one (trisomy). In summary, the Qploidy approach allowed us to characterize many samples in terms of ploidy, aneuploidy, and, in some cases, the parental origin of aneuploid gametes in mapping populations.

722S **Fine-scale genetic mapping using synthetically recombined chromosomes** Christopher J Ne Ville, Alessandro Venega Coradini, Ian Ehrenreich Molecular and Computational Biology Section, Department of Biological Sciences, University of Southern California

Since Mendel, geneticists have used crosses to characterize genotype-phenotype relationships. While crosses remain an

incredibly powerful tool in genetics research, they require mating, meiosis, and living parents, and often provide poor mapping resolution due to limited recombination during gametogenesis.

To enable the production of highly recombined chromosomes without crossing, we extendedCReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a technique that we previously developed for cloning segments of natural chromosomes and building synthetic chromosomes from them. In its prior iteration, CReATiNG exclusively involved in vivo DNA assembly of large chromosomal segments, limiting the amount of synthetic recombination that could be generated between two or more donor chromosomes. Here, we introduce an intermediate in vitro assembly step into CReATiNG, after cloning and before chromosome assembly. This step enables the efficient synthesis of chromosomes with substantially more recombination breakpoints and makes it scalable to generate synthetically recombined chromosomes with many, if not all, possible combinations of donor segments. To illustrate these points, we report our efforts to generate 512 recombinant versions of Chromosome I, a ~200-kilobase chromosome, between the budding yeasts Saccharomyces cerevisiae and S. paradoxus in an otherwise isogenic genetic background. This library of synthetically recombined chromosomes should allow us to map the genetic basis of interspecies trait differences at the resolution of 10-20 kilobases.

723S **Determining Gene Function of *YBR220C* in *Saccharomyces cerevisiae*** Hailey H Hendricks, Jill B Keeney Biology, Juniata College

The model yeast *Saccharomyces cerevisiae* is widely studied, yet the function of nearly 10% of genes is unknown; my research focuses on one of these genes of unknown function. I study *YBR220C*, a non-essential protein of unknown function with gene ontology terms of molecular function, biological process, and cellular component annotated as unknown. A series of bioinformatic modules revealed that *YBR220C* may be involved Acetyl-CoA transport and may be localized to the endoplasmic reticulum. In an attempt to determine the function of Y*BR220C*, I deleted the *YBR220C* ORF to study phenotypic differences from wild type in specific growth conditions. Deletion does not impact growth rate. Wild-type and *YBR220C* deletion strains were treated with a variety of carbon sources including 2-deoxy-D-glucose, glucose, acetate, ethanol, and glycerol. Results showed that these medias had no effect on the growth of a *YBR220C* deletion strain compared to wild-type. I›m currently investigating the cellular component of *YBR220C* through fluorescent tagging.

724S **Characterizing and cataloging *Saccharomyces cerevisiae* isolates from ancient metagenome samples** Megan Brown[1], Bryce Taylor[2] [1]Loras College, [2]Biology, Loras College

Saccharomyces cerevisiae, better known as brewer's yeast, has been a companion to humans for thousands of years. During this time, yeast have undergone a domestication process that has yielded strains specialized for baking bread, brewing beer, and making wine. We can see results of this domestication when we look at their genomes. A large body of research has identified genetic variation that likely contributed to the domestication process by providing yeast isolates with useful traits. However, we don't have a good understanding of the evolutionary history of S. cerevisiae, or how our modern strains came to be. One way that we can fill in this timeline is by investigating the genomes of yeast found in ancient samples like coprolites (fossilized feces) or fossilized dental calculus. I am cataloging and characterizing yeast from ancient metagenome studies to gain new insight into the domestication process.

My first aim characterizes a yeast that was identified in Iron Age coprolites from miners in Hallstatt, Austria by a group led by Frank Maixner. I aligned sequencing reads to a reference genome and called SNPs from this strain. I then compared these to a list of known S. cerevisiae SNPs from the 1,002 Genome Project. This left me with 525 candidate novel SNPs. At least three of these ancient SNPs are in genes associated with phenotypes that could be useful for food and beverage production (CPA2, HXK1, and SSA4). I will be using CRISPR/Cas9 to edit these SNPs into a modern yeast to see if they change the phenotype of the modern yeast. This will give us a better understanding of the properties of the Hallstatt yeast, filling in a new point on the evolutionary timeline.

My second aim is to curate a list of other ancient metagenome datasets that include Saccharomyces cerevisiae sequence reads. This information will allow scientists and archaeologists with an interest in fermentation to easily determine where yeast DNA was found in ancient samples. It will additionally enable researchers to investigate how yeast strains were shared among and migrated with ancient peoples.

725S **Examination of the genome of the species *Saccharomyces cerevisiae*** Fred S Dietrich Molecular Genetics and Microbiology, Duke University

Whole genome sequencing of strains of the species *S. cerevisiae* makes it possible to identify the core gene set, and the variable features found in the genome of this species. Using a definition of a gene being conserved at the same location and

orientation in 95% of genome sequences, this species has 5873 core protein coding and RNA coding genes. Analysis suggests more that 200 genes currently annotated in strain S288c should be relabeled as dubious, and there are less that 400 protein coding genes of unknown function. Careful analysis of the genomes identifies an unusual transposable element (Ty7), found in many strains but not in S288c. There are also gene remnants, pseudo genes, and non-core genes, those found in less than 95% of strains at the same location and orientation. Gene remnants are areas where in most or all strains examined homology could be detected to genes found in other organisms, though multiple frame shifts and stop codons suggest the gene is lost in this species. Comparison of the genomes reveals a very clear pattern of the central portions of the genome being comprised primarily of core genes, and a distinct location where the sub-telomere begins and many of the strain-specific genes and pseudogenes are found. Careful analysis of these genomes uncovers multiple interesting features.

Example 1: The COX5B gene contains an intron where the 5' splice site is GC. The other 277 nuclear encoded introns have GT at the 5' splice site, usually GTATGT. Several other introns are currently annotated in S288c as having GC at the 5' splice sites, but they all appear to be dubious introns. 94 out of 94 of the *S. cerevisiae* strains examined in this work, as well as the COX5B gene in *S. paradoxus* have GC at the splice site.

Example 2: Genes encoding tRNA's are generally quite conserved, though there is one example of a change in the anti-codon. While all strains contain multiple copies of a gene encoding identical alanine tRNA (AGC), four strains contain an unusual tRNA, possibly acquired from *Torulospora* sp. by introgression that appears to be an Alanine tRNA (AGC), but the tRNA sequence is similar to Threonine tRNA's. In *Torulospora*, there are two types of (AGC) tRNA genes, one type 100% identical to the Alanine to tRNA genes found in all strains, and the other very similar to the novel tRNA.

More details about this work can be found in bioRxiv 2023.09.07.545205 726S

Investigating the effects of yeast deletion mutations under a resource partitioning model of the cell Michael S. Overton, Sergey Kryazhimskiy Biological Sciences, University of California, San Diego

An important way that microbes adapt to new environments is by acquiring mutations that increase growth rate. However, mutations do not affect proliferation directly, but are transmitted through the molecular architecture of the cell, e.g. a nucleotide substitution changing an amino acid in a catalytic site, which goes on to alter the flux through a hierarchy of pathways to finally affect growth. While this architecture has proven difficult to parse, certain features of biological growth may reduce its complexity, such as its intimate reliance on nutrient metabolism and protein synthesis through translation. A recently developed proteome resource partitioning model (PRPM) exploits these features to accurately predict bacterial growth rates under varying environments using only a few parameters. These include the relative sizes and catalytic efficiencies of proteome sectors devoted to nutrient metabolism and ribosome biosynthesis and the proteome fraction devoted to other processes. To test whether this model is also applicable to the effects of mutations, we reanalyzed yeast transcriptomic and growth rate data of 650 deletion mutants from a pair of recent studies. We found that mutations perturbed the transcriptome in strikingly similar patterns to those expected under the PRPM. Specifically, expression profiles clustered transcripts into three groups, each enriched for GO terms associated with one of the three PRPM sectors. Expression levels of the putative nutrient and ribosome sectors had a strongly negative, linear association, while the third, putative housekeeping sector remained relatively unperturbed. In addition, the growth rate effects of mutations were positively associated with ribosome sector size. These results are all consistent with the PRPM, and suggest that mutations can shift resource allocation between the ribosome and nutrient sectors, which may, in part, explain their growth rate consequences. However, mutations also tended to affect the catalytic efficiencies of the nutrient and translation sectors jointly, rather than independently, as would be expected from the physiological modularity assumed under the PRPM. This suggests that cellular physiology responds to mutations in a manner distinct from that of environmental perturbations. These contrasting results beg for further characterization of yeast physiology under the PRPM, as it could provide a simple and generic mechanism whereby mutations produce growth rate changes.

727S **High-throughput Screening for Aflatoxin B1 Resistance Genes in Budding Yeast and VERO-E6 Cells Identifies Genes That Confer Resistance to Replication Stress** Michael Fasullo[1], Chris Vulpe[2], Abderrahmane Tagmount[2], Miral Dizdaroglu[3], Bharti Bharti[4] [1]Nanoscale Science and Engineering, University at Albany, [2]University of Florida, [3]National Institute of Standards and Technology, [4]University at Albany

Introduction: Aflatoxin B1 (AFB1) is a class I carcinogen and a potent known hepatocarcinogen. The bioactivated epoxide derivative generates mutagenic DNA adducts. Previously, we used a "humanized" yeast deletion library that expresses CYP1A2 to profile the yeast genome and identified 86 resistance genes, which included 15 DNA repair genes. These DNA repair genes included those that participate in nucleotide excision repair (NER) and homologous recombination. We hypothesize that mammalian genes, orthologous to these yeast genes, also confer AFB1 resistance. **Methods:** Our strategy to identify mammalian AFB1 resistance genes utilizes a CRISPR KO approach to knockdown ~19,053 genes in green monkey kidney

epithelial (VERO-E6) cells. Four sgRNAs for each of the ~19,053 genes were cloned into the LentiCRISPRv2 Puro vector to generate the CRISPR KO library. The resulting transfectants were exposed to 200 nM AFB1 for seven and fourteen days. AFB1-associated DNA adducts were detected after exposure to 200 nM AFB1. Resistance and sensitive genes were identified using the negative binomial approach implemented in edgeR and CRISPR analysis software (MAGeCK). Data sets were obtained at t0, and at t7 and t14 for toxicant exposed and control cells. **Results:** From comparing toxicant exposed (t7, t14) and the initial cell library (t0), we identified 55 common sensitivity genes, including genes involved in AFB1 bioactivation (POR, CYP3A4) and senescence (SMARCD1, BACH1, CDKN1). We identified twelve common resistance genes, including those that function in DNA replication stress (RFC3, MCM3) and in non-homologous end joining (APLF). In comparing toxicant (t7) to control (t7), we identified NER genes (ERCC4, UVSA). XRCC3, a Rad51 paralog, was identified in comparing t14 to t0 data sets. **Discussion**: We obtained initial results that genes conferring AFB1 sensitivity in VERO include those that bioactivate AFB1, and genes conferring resistance include NER NHEJ, and genes that participate in homologous recombinaiton. These data suggest that a subset of DNA repair pathways that confer AFB1 resistance are shared among both lower and higher eukaryotes.

728V **NCBI's RefSeq Select dataset for mouse genome** Anjana R Vatsan, Alexander Astashyn, Olga Ermolaeva, Tamara Goldfarb, Diana Haddad, John Jackson, Vinita Joardar, Vamsi Kodali, Kelly McGarvey, Michael Murphy, Craig Wallin, David Webb, Terence D Murphy, Shashikant Pujar National Center for Biotechnology Information of the National Library of Medicine, National Institutes of Health

The reference sequence (RefSeq) project at the National Center for Biotechnology Information (NCBI) aims to provide high quality, complete and accurate annotation of human and mouse genomes along with other organisms across the tree of life. Genes often are annotated with multiple RefSeq transcripts and proteins due to alternative splicing. While some alternatively spliced transcripts have been shown to play important functions in several tissues including disease related state, others may not be of great significance. Several transcripts per gene can also become problematic in certain studies like comparative genomics and evolutionary analysis. NCBI's RefSeq Select dataset provides a representative transcript for every protein coding gene. RefSeq Select transcripts are well supported by experimental data, biologically relevant and conserved. The dataset is produced using computational processes and manual curation by a group of expert curators. The RefSeq Select dataset was originally available for human but now includes mouse (GRCm39) and rat (mRatBN7.2) genomes, as well as a subset of prokaryote proteins. We aim to expand it to other high value organisms. The RefSeq Select transcripts can be accessed from multiple NCBI resources such as Gene, RefSeq, Genome Data Viewer and annotation files, and are available for bulk download from NCBI's FTP sites. BLAST now offers RefSeq Select databases for faster searches and better results. This work is funded by the National Center for Biotechnology Information of the National Library of Medicine, National Institutes of Health.

729V **Genotype vs environment effect on housefly *(Musca domestica)* microbiome** Sohana Al Sanjee, Kiran Adhikari, Richard P. Meisel Biology and Biochemistry, University of Houston

Host-associated microbiomes affect physiology, behavior, health, and other phenotypes. Microbiomes can be highly variable among individuals, which can be caused by genetic differences between hosts, variation across environments, or the interactions between genotype and environment. Temperature may be an especially important environmental factor on host-associated microbiomes because it affects both host physiology and myriad bacterial characteristics. In particular, increased temperature can disrupt the microbiomes of ectotherms, but it is not clear if that disruption is caused by higher temperatures *per se* or merely by deviating from the thermal optimum. Addressing that question requires shifting a warm adapted host to a cooler temperature and measuring the change in the associated microbial community. House fly (*Musca domestica*) is a well-suited model organism to those ends because it is warm-adapted animal with segregating genetic variation that modulates temperature-dependent phenotypes. We performed two studies of house fly associated bacterial communities across genotypes and environments. First, we used 16S rRNA sequencing to profile the microbiomes of two-house fly genotypes with temperature-dependent phenotypic effects raised at two different temperatures (18°C and 29°C). We sampled 26 male flies from each of the genotype-by-temperature combinations. Second, we sequenced 16S rRNA from 86 house fly males from two collection sites in Texas. The bacterial communities associated with the house flies differed between the environments in both studies (either developmental temperature or collection site), but we did not detect significant differences between genotypes. Specifically, the house flies raised at 29°C had microbiomes that were more similar to each other (i.e., lower between-fly beta diversity) than the 18°C flies. Similarly**,** there were differences in the between-fly bacterial diversity between the two collection sites within Texas. Our results therefore provide consistent evidence of an environmental effect on the house fly microbiome and no evidence of an effect of the host genotype. Our results also provide evidence that colder temperatures increase the heterogeneity of the house fly microbiomes. More generally, our results suggest that deviation from the thermal optimum (in this case shifting from warm to cooler temperatures) disrupts the host associated microbiome, regardless of whether the disruption is toward hotter or colder temperatures.

730V **Sequence Analysis of Heat Resistant Gene in Naked Neck Nigerian Indigenous Chicken** Hannah E Etta Animal and

Environmental Biology, University of Cross River State

The sequence characteristics of heat resistant genes (HRG) in naked neck Nigerian Indigenous chicken (NIC) was investigated. The HRGs are IL6, HSPB2, HSP2B, HSFP, and HSP90. Relevant DNA and amino acid sequences for the heat resistant genes hoisted by the National Centre for Biotechnology Information (NCBI) database were sourced for and retrieved from the genebank using the FASTA format and basic local alignment search tool (BLAST). The were analyzed using Chromas 7.0 software. The results revealed a total of 10 conserved regions which were visible at the exons sites of the multiple aligned nucleotide sequences of the heat resistant genes. Results obtained for Protein sequence characterization showed high variability in the characteristics of heat resistant genes. The polymorphic characteristics scores were also obtained. From these results, it can be deduced that the HSG in the naked neck NIC should be preserved as it shows low mutation rate and can also be used to modify the genes of other organisms that are very susceptible to high temperature especially here in the tropics. This investigation presents valuable insights into the functional roles of the HRG in thermal adaptation, stress response, and overall health Status of the naked neck NIC. It provides bench mark information that could be useful in the strategic planning for the conservation and management of the breed.

731V **Three-dimensional genome organization in the mosquito central nervous system** Christopher Catalano[1], Xiao Li[2], Michael Levine[2] [1]Molecular Biology, Princeton University, [2]Lewis-Sigler Institute, Princeton University

Mosquito-borne diseases are responsible for nearly one million deaths per year, killing more humans than any other predator by far. There have been intensive efforts to understand and modify the genomes of disease-bearing mosquito species, and here we add to the understanding of the chromosome-level organization of the mosquito genome. Recently described meta-loops in *Drosophila* coordinate the expression of distant genes, spanning 2-20 megabases, primarily in post-mitotic neurons of the central nervous system. We have produced a comprehensive Micro-C chromosome conformation map for the adult brain of the malaria vector, *Anopheles coluzzii*. This high-resolution, three-dimensional map reveals long-range meta-loops in the adult *Anopheles* brain. These meta-loops are often associated with genes influencing neuronal function, including cell surface adhesion molecules involved in axon guidance and connectivity. Further, we have identified specific DNA segments that act as robust loop anchors, facilitating spatial interactions between distant genomic loci. This work has the potential to identify regulatory loops as promising targets for disruption, adding to the arsenal of technologies aimed at controlling mosquito behaviors in natural populations.

732V **5-Nitroimidazole-resistance in *Mycoplasma genitalium* strains is associated with mutations in an NAD(P)H-dependent FMN reductase gene.** Abhi Kancherla[1], Alessandro Rizzi[2], Cameron Weller[2], Derek Wood[3], Gwendolyn Wood[4] [1]Seattle Pacific University, [2]Department of Chemistry and Biochemistry, Seattle Pacific University, [3]Department of Biology, Seattle Pacific University, [4]Department of Medicine, University of Washington

Mycoplasma genitalium (MG) is a sexually transmitted pathogenic bacteria that causes urethritis in men and pelvic inflammatory disease (PID) in women. MG has developed resistance to first-line antibiotics like azithromycin, doxycycline, and moxifloxacin—making it one of three microorganisms added to the CDCs Watch List for Antibiotic Resistant Threats in the US. 5-Nitroimidazoles (5-Ni) are being evaluated as alternative treatment options based on two recent studies. A 2020 study by Weisenfield found that adding metronidazole to a standard multidrug treatment with ceftriaxone and doxycycline reduced MG presence in patients with PID and we recently showed that MG was susceptible to 5-Nis. These drugs may provide alternative treatment options following clinical studies, however, MG can develop resistance to them. In this study, we isolated and sequenced the genomes of nine 5-Ni-resistant G37S mutants with the MinION Mk1B genome sequencer. We identified mutations in the MG_342 and MG_179 genes, which encode for a putative NAD(P)H-dependent FMN reductase and energy-coupling factor (ECF) transporter ATPase, respectively. Mutations in the FMN reductase may cause reduced expression or loss of function of the enzyme leading to resistance.

733V **Investigating the Role of Transposable Element-Derived Promoters in Tissue-Specific Gene Expression and Phenotype in Zebrafish Testis** Irene Wang, Yujie Chen, Hyung Joo Lee, Ju Heon Maeng, Yiran Hou, Ting Wang Washington University School of Medicine in St. Louis

Transposable elements (TEs) are repetitive sequences found in various eukaryotic genomes, constituting a substantial portion of vertebrate genomes, including zebrafish. Emerging studies suggest that TEs can act as cis-regulatory elements, offering alternative promoters for gene regulation. Previous work from our lab demonstrated that 37% of TEs are associated with active regulatory states in adult zebrafish tissues, particularly in the testis. Additionally, we confirmed that novel TE-derived promoters have the capacity to initiate testis-specific transcription of alternate gene isoforms. However, it remains unclear whether these TE-derived promoters play a crucial role in testis development.

In this study, we have selected two promising candidates, *srl* and *rasgrp4*, from a pool of 413 novel TE-derived promoters identified using nanoCAGE and RNA-seq analyses. Employing CRISPR-Cas9 technology, we performed targeted deletion of both the canonical promoter and the TE-derived promoter for each candidate. Our goal is to generate homozygous mutant zebrafish lines to delve into the functional significance of TE-derived promoters in terms of their impact on testis-specific gene expression and overall testis development. This investigation will shed light on the essentiality of TE-derived promoters in zebrafish development and tissue-specific gene regulation.

734V **Health assessment of Resident Killer Whale populations via whole genome analysis** Lance Barrett-Lennard[1], Adam Warner[1], Stephane Flibotte[2], Thomas Doniol-Valcroze[3], Sheila Thornton[3] [1]Raincoast Conservation Foundation, [2]LSI Bioinformatics Facility, University of British Columbia, [3]Fisheries and Oceans Canada

Southern Resident Killer Whales (SRKW) and Northern Resident Killer Whales (NRKW) are listed as endangered and threatened, respectively, under Canada's Species at Risk Act, and recovery of these populations face a number of obstacles. Noise impacts on Resident Killer Whale (RKW) individuals and populations include degradation of the acoustic environment, stress responses, and a decrease in foraging efficiency. In addition, displacement of RKW from key foraging areas is likely to result in changes in the stock composition of their salmonid prey and may lead to nutritional deficiencies. Hormone analyses from individuals (fecal and blow) provide insights into the impacts of both noise and nutritional stress, and identification of prey from fecal samples informs our knowledge of prey selection and foraging success. Health-related hormone data can be linked to a specific individual by genotyping the RKW DNA found within fecal samples and matching to archived skin biopsy samples from RKWs.

While anthropogenic impacts are of primary concern, of equal importance is the need to identify intrinsic factors that may be limiting SRKW recovery. This includes the extent of inbreeding and its impacts on the population. Comparisons of SRKW inbreeding to that observed in the threatened NRKW population is an important aspect of this analysis, as this small ecologically- similar population continues to increase in numbers. Whole genome sequences obtained from 141 NRKW biopsies collected over the last 25 years have been used to assess the level of inbreeding within the population, and comparisons with the SRKW data reveal the level of genetic diversity within each of the populations. Characterizing the impact of intrinsic factors such as inbreeding is necessary to obtain a more complete understanding of the magnitude of anthropogenic threats. This knowledge supports the development of efficient and effective mitigation measures targeted at anthropogenic threats.

In addition, unique genetic fingerprints have been produced for each of the 141 NRKW in this study using a 68 loci SNP panel to provide as a reference for matching RKW fecal samples collected for health-related hormone analysis to specific individuals. We have developed a Nanopore-based SNP amplicon sequencing protocol to rapidly genotype RKW fecal samples using the same 68 loci SNP panel, allowing for in-house DNA sequencing and matching of fecal samples using commercially available flow cells. Matching of RKW fecal samples to individuals allows for health-based assessments to be linked to individuals, and a broader understanding of overall population health.

735T **Extreme allelic diversity likely underlies adaptation to environmental heterogeneity** Erik Andersen Biology, Johns Hopkins University

Starting in the 1950s, Stebbins and others argued that selfing is an evolutionary dead end because these species cannot adapt to changing environments. However, countless selfing species have persisted over long evolutionary time scales, so they must have adapted to environmental heterogeneity. Recently, numerous examples of punctuated genomic regions harboring high levels of diversity have been discovered across selfing lineages of plants and nematodes. In the *Caenorhabditis* genus, selfing has evolved three times independently, and these hyper-divergent regions are found in all three species. Specifically in *C. elegans*, these regions were found to contain unique sets of genes enriched for environmental response functions, including pathogen resistance, stress responses, and food sensing. These discoveries led to the hypothesis that hyper-divergent regions could give these selfing species a means to adapt to changing environments. We used short- and long-read technologies to create new reference genomes and gene models for *C. briggsae* and *C. tropicalis*, the two less well studied species as compared to *C. elegans*. Additionally, we sequenced the genomes of all known wild strains from each of these three species to identify hyper-divergent regions and their gene contents. We have found hundreds of previously unknown and uncharacterized *Caenorhabditis* genes, and these genes are enriched for environmental responses. These wild strains paired with ecological sampling data led to hypotheses about adaptation to specific environmental stress (*e.g.*, pathogens). I will present our progress validating how the genes in these regions promote adaptation to different stresses in the natural niche. The evolutionary origins of these regions are unknown but could be ancestral genetic diversity maintained by long-term balancing selection from an outcrossing ancestor or recent adaptive introgression from a closely related outcrossing species. Because *C. elegans* lacks a closely related outcrossing species, we turned to *C. briggsae* and its closely related outcrossing

sister species, *C. nigoni*, where we can investigate signatures of balancing selection or evidence of recent introgression events. We sought to identify shared genetic variation and haplotypes between these two species to test hypotheses about the origins of hyper-divergent regions. The results from these studies provide insights into how selfing shapes the patterns of diversity in selfing species and how selfing species are able to adapt to environmental heterogeneity.

737T **Rapid evolutionary diversification of the *flamenco* locus across simulans clade *Drosophila* species** Sarah Signor[1], Jeffrey Vedanayagam[2], Bernard Kim[3], Filip Wierzbicki[4], Robert Kofler[4], Eric C. Lai[5] [1]North Dakota State University, [2]University of Texas, San Antonio, [3]Stanford University, [4]Vetmeduni Vienna, [5]Sloan-Kettering Institute

Suppression of transposable elements (TEs) is paramount to maintain genomic integrity and organismal fitness. In *D. melanogaster*, the *flamenco* locus is a master suppressor of TEs, preventing the mobilization of certain endogenous retrovirus-like TEs from somatic ovarian support cells to the germline. It is transcribed by Pol II as a long (100s of kb), single-stranded, primary transcript, and metabolized into ~24-32 nt Piwi-interacting RNAs (piRNAs) that target active TEs via antisense complementarity. *flamenco* is thought to operate as a trap, owing to its high content of recent horizontally transferred TEs that are enriched in antisense orientation. Using newly-generated long read genome data, which is critical for accurate assembly of repetitive sequences, we find that *flamenco* has undergone radical transformations in sequence content and even copy number across *simulans* clade Drosophilid species. *Drosophila simulans flamenco* has duplicated and diverged, and neither copy exhibits synteny with *D. melanogaster* beyond the core promoter. Moreover, *flamenco* organization is highly variable across *D. simulans* individuals. Next, we find that *D. simulans* and *D. mauritiana flamenco* display signatures of a dual-stranded cluster, with ping-pong signals in the testis and/or embryo. This is accompanied by increased copy numbers of germline TEs, consistent with these regions operating as functional dual-stranded clusters. Overall, the physical and functional diversity of *flamenco* orthologs is testament to the extremely dynamic consequences of TE arms races on genome organization, not only amongst highly related species, but even amongst individuals.

738T **An Evolutionary Puzzle: Examining the High Insertion Bias of P-Elements into X-TAS** Shashank Pritam[1], Robert Kofler[2], Almorò Scarpa[3], Sarah Signor[1] [1]Biological Sciences, North Dakota State University, [2]Institut für Populationsgenetik, [3]Vienna Graduate School of Population Genetics

Transposable elements, also known as transposons or “jumping genes,” are DNA sequences that can move and replicate within the genome. Transposons significantly impact genome stability, and understanding their dynamics is crucial for evolutionary biology and medical research. The formation and function of piRNA clusters still need to be fully understood. Previous research based on the prevalent population genetics model called the “Trap Model” has demonstrated that invading transposable elements (TEs) are stopped in their proliferation when a TE copy gets inserted into a piRNA cluster. This insertion triggers the production of piRNAs that silence transposons in the germline. The P-element, a well-known piRNA cluster, has been observed to show a strong insertion bias into piRNA clusters. This is peculiar behavior since piRNA clusters are known to silence transposable elements (TEs) according to the Trap Model. To understand the dynamics of transposon insertion and its influence on genome stability, we investigated transposon dynamics accounting for piRNA-mediated silencing of TEs. Our preliminary result suggests that organisms can compensate for smaller piRNA cluster sizes through increased transposon insertion bias into the clusters. Population fitness rises with greater cluster size and higher average insertion bias. However, negative bias leads to population extinction despite the large cluster size. Further research quantifying transposon impacts and insertion biases across species will enable more accurate modeling of this arms race between transposons and host genomes. Our model provides a framework for understanding transposon invasion and the role of piRNAs in maintaining fertility and genome stability.

739T **Evolutionary rate covariation is pervasive between unrelated glycosylation pathways and points to potential disease modifiers** Holly Thorpe[1], Nathan Clark[2], Clement Chow[3] [1]Human Genetics, University of Utah, [2]University of Pittsburgh, [3]University of Utah

Glycosylation, a common post-translational modification, is necessary for protein localization, folding, and stability. Defects in glycosylation pathways, such as N-linked glycosylation, O-linked glycosylation, and GPI anchor synthesis, lead to rare diseases classified as Congenital Disorders of Glycosylation (CDG). CDGs typically present with seizures, hypotonia, and developmental delay, but display large clinical variability with symptoms affecting every system in the body. This variability suggests modifier genes affect the phenotypes. I am employing evolutionary approaches to identify modifier genes of CDGs.

Evolutionary Rate Covariation (ERC) relies on the premise that proteins that interact physically or genetically or are functionally related evolve at similar rates. ERC values are calculated using the correlation coefficient of evolutionary rates of protein pairs in a species tree. I used ERC to identify genome-wide covariation with proteins involved in GPI anchor synthesis. There was enriched, strong ERC among GPI anchor synthesis proteins. Unexpectedly, there was also enriched ERC between GPI anchor

synthesis proteins and proteins in other glycosylation pathways, suggesting underappreciated overlap between the different pathways. Gene Ontology analysis of top proteins showing high ERC with GPI anchor synthesis proteins revealed enrichment in RNA modification and DNA damage repair, suggesting interactions between these processes and GPI anchor synthesis. Protein pairs with the highest ERC scores included HTT and PIGG and ATG7 and PIGG. HTT and ATG7 are associated with neurodegenerative disorders, possibly indicating overlap in pathophysiology between the disorders.

To functionally validate these exciting signals, I tested for genetic interactions using the *Drosophila* eye as a model. Many GPI anchor synthesis genes are necessary for *Drosophila* eye development and knockdown of these genes leads to small, disorganized, rough eyes. Using double knockdowns of GPI anchor synthesis genes and coevolving genes in the *Drosophila* eye, I identified genetic interactions between genes previously thought to be unrelated. Many of the strongest evolutionary signals validate as interactors in this *in vivo* analysis. Evolution and covariation are underutilized tools for identifying disease-relevant, novel genetic interactions. These connections could lead to better understanding of glycosylation pathways and potential treatments for CDGs.

740T **Population genomics of *Drosophila pseudoobscura*** Camryn Kritzell, Zhao Zheng, Christopher Gonzales, Richard Meisel University of Houston

Drosophila pseudoobscura is a model organism for population genetics, speciation genetics, and sex chromosome evolution. For example, natural populations of *D. pseudoobscura* harbor a rich inversion polymorphism on their third chromosome, which has been a model system for studying clinal variation and genome evolution for decades. In addition, *D. pseudoobscura* has a neo-X chromosome, which formed when an autosome fused to the ancestral *Drosophila* X chromosome. This neo-X chromosome represents a natural evolutionary experiment that is informative of the selection pressures acting on X-linked genes. Despite the tremendous utility of *D. pseudoobscura* as a model organism, genome-wide genetic variation has yet to be comprehensively explored in this species beyond the third chromosome inversion polymorphism. To address that shortcoming, we sequenced the genomes of >60 isolates (inbred lines) of *D. pseudoobscura* from across the species' range in North America. We used these data to identify single nucleotide polymorphisms and small insertion/deletion polymorphisms, as well as fixed substitutions with a closely related species (*Drosophila miranda*). Tajima's *D* was negative across most of the genome, consistent with a recent population expansion. However, the magnitude of Tajima's *D* varied across chromosomes, suggesting unique intra-specific evolutionary trajectories across chromosomes. We found no evidence for population structure across most of the *D. pseudoobscura* genome, consistent with previous analyses of individual loci that suggested high migration rates across the species' range effectively create a single, panmictic population. In contrast, there was substantial evidence for population structure on the third chromosome, which we attribute to inbred lines carrying different arrangements of the third chromosome. We used this population structure, along with geographic information, to probabilistically infer the arrangements carried by each line. Lastly, we failed to detect evidence for a higher adaptive substitution rate on the ancestral- or neo-X chromosomes, suggesting that there is not a faster-X effect.

741T **Accelerated evolution in the protein components of the genomic dark matter, heterochromatin** Leila Lin[1], Yuheng Huang[1], Jennifer McIntryre[1], Ching-Ho Chang[2], Serafin Colmenares[3], Yuh Chwen G Lee[1] [1]Ecology & Evolution, UC Irvine, [2]Fred Hutchinson Cancer Research Center, [3]Cell and Molecular Biology, UC Berkeley

Heterochromatin plays an important role in maintaining the stability of the genome despite being the transcriptionally silent, gene-poor, and repeat-rich compartment of the genome. Without the silencing effects of heterochromatin, deleterious selfish genetic elements, such as transposable elements, are free to jump around the genome and disrupt functional elements. Accordingly, genes that encode the regulatory elements that modulate heterochromatin are theorized to be highly conserved due to their essential functions. However, previous studies have found a few heterochromatin-regulating genes fast evolve through rapid accumulation of amino acid substitutions or exhibit differences in gene copy number between species. Here, we aim to investigate if previously observed fast evolution of genes modulating heterochromatin is a common trend among all genes known to influence heterochromatin function amongst 16 *Drosophila* species, spanning 40 MYR. Our candidate genes include (1) PEV modifiers known to modulate the spreading of repressive epigenetic marks from heterochromatin, (2) histone-modifying enzymes that influence enrichment levels of repressive epigenetic marks, H3K9me2/3, and (3) genes whose protein products localize to heterochromatin, suggesting their potential functional roles in heterochromatin. For the control, we used polycomb genes as they also interact with repressive epigenetic marks, H3K27me3, but play no role in heterochromatic function. We found that, compared to control polycomb genes, candidate genes that enhance heterochromatin silencing show more prevalent adaptive evolution in terms of changes in amino acid sequences and copy number variation at both short and long evolutionary time scales. Many of the signatures of accelerated evolution fall within domains with known functions, such as nucleic acid binding and histone modification. Interestingly, evidence of adaptive evolution was also found in intrinsically disordered regions (IDRs) that are suggested to play important roles in the phase properties of heterochromatin. In fact, we found that fast-evolving candidate genes show greater variability in the total IDR content than other genes. We further

identified that the evolutionary rates of our candidate genes are significantly associated with the abundance of repetitive sequences across species. These findings suggest that the rapid evolution of genes involved in heterochromatin regulation may be tightly intertwined with the rapidly changing repeatome through not only their well-structured protein domains but also their phase properties.

742T **Breaking mating barriers between *Drosophila melanogaster* and *D. simulans*** Tianzhu Xiong, Daniel Barbash Molecular Biology and Genetics, Cornell University

Interspecific hybrids between *Drosophila melanogaster* and *D. simulans* have been incredibly useful to study repetitive DNA regulation and hybrid incompatibilities. However, creating hybrids is difficult, especially between *D. melanogaster* males and *D. simulans* females. Here we report the progress to improve the mating rates in this cross direction. Between 15% to 43% of females can be successfully inseminated by the other species using our methods, with variation attributed to different female strains. The improvement in mating rates enables us to screen for polymorphic maternal factors of *D. simulans* causing variable levels of hybrid lethality.

743T **Drosophila tripunctata: Is sympatric speciation occurring due to host preference and toxin tolerance ?** Grace Kropelin, Clare Scott Chialvo Biology, Appalachian State University

An evolutionary arms race between insects and hosts is no new concept. For millions of years, they have been in this evolutionary race that results in the evolution of host defense mechanisms and adaptations in the insets to mitigate the risks of the host defenses. One example of a defensive product includes producing highly, toxic compounds. These compounds deter most insects, typically those classified as generalist feeders. Generalist feeders have not developed the strategies to combat these toxic compounds. Despite this, some mushroom-feeding *Drosophila* species, considered to be generalists, have been shown to be tolerant of deadly cyclopeptide mushroom toxins. Specifically, species within the *immigrans-tripunctata* radiation including our species of interest, *Drosophila tripunctata*. Previous studies have shown that *D. tripunctata* tolerates the deathcap mushroom cyclopeptide toxins. Furthermore, *D. tripunctata* uses both mushrooms and tomatoes as hosts and possesses significant genetic variation for toxin tolerance. Due to their host choice and toxin tolerance to the deathcap mushroom, we hypothesize that *D. tripunctata* is potentially undergoing sympatric speciating. To assess this, we are examining host preference and toxin tolerance in a population of 101 genetically unique inbred lines of *D. tripunctata*. In addition, we sequenced the genome of each line to identify single nucleotide polymorphisms associated with both host preferences and mushroom toxin tolerance. Our results will increase our understanding of how traits associate with a novel adaptation typically found in specialist feeders impacts a generalist species.

744T **Integrating genomes and legacy marker data to estimate the Drosophilidae Tree of Life** Bernard Y. Kim[1], Hannah R. Gellert[1], Samuel H. Church[2], Ben Hopkins[3], Richard Xu[4], James A. Hemker[1], Sofia G. Beskid[1], Patrick O'Grady[5], Masanori J. Toda[6], Artyom Kopp[3], Thomas Werner[7], Dmitri A. Petrov[1], Anton Suvorov[4] [1]Stanford University, [2]Yale University, [3]University of California Davis, [4]Virginia Tech, [5]Cornell University, [6]Hokkaido University, [7]Michigan Technological University

The family Drosophilidae is a premier model system for studies in biology, including genomics, ecology, and evolution. Resolving the phylogenetic relationships of the >4,400 described species in the family is of fundamental importance for interpreting these studies through the lens of evolutionary processes. Despite this, many of the phylogenetic relationships in this model family remain unresolved. So far, the majority of genomic data acquisition efforts were concentrated around the model genus *Drosophila*, which includes species that have played a fundamental role in life science research. This taxonomic bias, where genomic data for the remaining taxa outside *Drosophila* are unavailable or highly fragmented, hindered progress in building the Tree of Life for the whole family Drosophilidae. With the recent advances of long-read sequencing technologies it became possible to affordably and efficiently generate high quality genome assemblies necessary for robust phylogenomic inference. Here, we gather, consolidate and combine large amounts of heterogeneous sequencing data, namely molecular markers and multi-locus datasets derived from newly assembled genomes to estimate phylogenetic relationships within the Drosophilidae family. This practical approach has a twofold advantage: (i) to drastically broaden taxon coverage across the entire family and (ii) to increase reliability of an estimated tree topology.

Using this procedure we inferred a highly resolved and stable phylogeny of the Drosophilidae family that includes >1,500 species across 38 genera. Additionally, we performed nodal-based divergence time estimation using several fossil calibration points. Such a dated phylogenetic tree represents an important resource for the communities of evolutionary biology. First, it serves as a prime structure to approach questions in micro- and macro-evolutionary research. Second, it exhibits an immense technical importance for multiple sequence alignment estimation, ancestral reconstruction and many other tasks.

745T **The co-evolutionary network of 155 species of *Drosophila*** Andrius Jonas Dagilis[1], Daniel Matute[2] [1]University of Connecticut, [2]University of North Carolina at Chapel Hill

Using 2470 genes among 155 species of *Drosophila*, we describe genes that evolve at more similar rates than expected by chance alone. This co-evolutionary network provides a resource to test a variety of long-standing evolutionary hypotheses, including the enrichment of co-evolution in co-localized genes. The co-evolutionary network of *Drosophila* is enriched for interactions among genes that are located on the same Muller elements, except for a notable decrease in the number of interactions among genes located on the *Drosophila melanogaster* X chromosome. We further investigate how these interactions relate both to protein interactions and known incompatibilities in *Drosophila*, and demonstrate how co-evolving genes may be more likely to co-introgress. Finally we compare the co-evolutionary networks of major clades of *Drosophila* to examine the stability of long-term evolutionary interactions.

746T **Clade-scale genomic data reveal heterogeneity in natural selection across the drosophilid protein-coding genome** Bernard Y Kim[1], Hannah R Gellert[2], Sofia G Beskid[2], Donald K Price[3], Thomas Werner[4], Darren J Obbard[5], Dmitri A Petrov[2] [1]Biology, Stanford University, [2]Department of Biology, Stanford University, [3]Life Sciences, University of Nevada, Las Vegas, [4]Biological Sciences, Michigan Technological University, [5]Institute of Ecology and Evolution, University of Edinburgh

The genome is heterogeneous in functional constraint down to the base pair level. The functional constraint evolves between species and these evolutionary shifts are indicative of the functional differences between species. Population genetic data – containing younger, mostly ephemeral variants that are less likely to be driven by positive selection affecting divergence between species – are the most useful kind of genomic variation data for quantifying constraint in a species and for understanding these shifts. Unfortunately, polymorphism is sparsely distributed throughout the genome, even in large datasets of high diversity species like *Drosophila melanogaster*. This leads to our inability to define constraint reliably even at the resolution of single genes, not to say anything at the resolution of specific codons within genes or specific short regulatory regions. To address this, we present an approach that quantifies constraint at very high resolution by integrating population genomic data across multiple closely related species. Specifically, we employed Oxford Nanopore long-read sequencing to rapidly generate over 300 inexpensive but high quality reference genomes for the model family Drosophilidae, along with population genomic resequencing datasets for ~150 of these species (~50 from a single wild individual), with the goal of generating genomes and population genomic datasets at the same scale. Then, we used whole-genome alignments to perform comparative population genomic analysis of variation at orthologous protein-coding positions across these 150 drosophilid species. This reveals extensive heterogeneity in the distribution of fitness effects and the impact of natural selection on genetic variation not only at the level of single genes, but even at resolutions of just a few amino acids. Remarkably, some proteins are evolving in a manner consistent with the strict neutral theory of molecular evolution, some evolve in accordance with the nearly neutral theory, and some show extremely high rates of adaptation. Furthermore, this heterogeneity is clearly related to the function and structure of the protein. The fine-mapping of natural selection we present here holds great promise for understanding the relationship between biological function and molecular evolutionary change and for connecting micro- and macro- patterns of molecular evolution.

747T **Fitness consequences of sex chromosome non-disjunction in *Drosophila melanogaster*** Nathaniel Sharp[1], Elizabeth Makovec[2], Caitlin Kestell[3], Kayla Janke[1], Ethan Carter[4], Aaron Ragsdale[1] [1]Genetics, University of Wisconsin-Madison, [2]University of Toronto, [3]North Carolina State University, [4]Xavier University

Non-disjunction of the sex chromosomes in *Drosophila melanogaster* provided foundational evidence for the chromosome theory of heredity over a century ago. Many subsequent studies using this system have detailed how chromosomes segregate, but the phenotypic and evolutionary consequences of such spontaneous mis-segregation events have not been well characterized. Non-disjunction can result in flies with an aneuploid XXY karyotype, which are females. While the Y chromosome has few protein-coding genes, its size and repetitive nature are known to contribute to genome-wide gene expression patterns in XXY females. This karyotype can potentially persist in a population, with XXY flies producing both XXY daughters and XYY sons. We address two related questions about this phenomenon. First, what are the phenotypes of XXY and XYY flies in terms of fitness-related traits? Second, how common are such flies in a laboratory population? We used visible markers to track the spontaneous appearance of XXY females and measured several phenotypes. XXY females showed normal viability and attractiveness, but also elevated size and fecundity, particularly when they were of spontaneous origin, as opposed to being derived from other XXY females. We performed a series of assays to rule out a negative maternal effect of XXY, indicating a parent-of-origin effect: females appear to benefit from having a paternally-derived Y chromosome, possibly due to imprinting. The higher fecundity of XXY females is offset by a slightly slower development time, and the production of smaller eggs. In contrast, while XYY males showed normal reproductive success, they also had reduced viability. We examined the standing frequency of XXY females in a large outbred laboratory population using both PCR and test crosses, finding a frequency of 1.3%. Given the apparently good fitness of XXY females, we used analytical models and simulations to understand why the standing frequency of XXY is not higher. We conclude that the XXY karyotype does not spread because of viability selection on XYY males, and that the XXY karyotype itself is neutral or beneficial. Our results shed light on the consequences of aneuploidy in an important model organism and have implications for genome evolution. Evidence has emerged that a natural

population of *D. melanogaster* recently evolved an XXY karyotype, indicating that our observations can help us understand chromosomal variation in both natural and laboratory populations.

748T **Origination and function of a newly identified female sexual behavior in *Drosophila santomea*** Shengxi Chen[1], Minhao Li[1], Ian P Junker[1], Fabianna Szorenyi[1], Guan Hao Chen[1], Arnold J Berger[1], Aaron A Comeault[2,3], Daniel R Matute[3], Yun Ding[1] [1]Department of Biology, University of Pennsylvania, [2]School of Natural Sciences, Bangor University, [3]Biology Department, University of North Carolina at Chapel Hill

Pre-copulatory sexual behaviors form an integral part of social interaction. In *Drosophila*, males often perform complex courtship behaviors that undergo rapid diversification, but female sexual behaviors and their evolution remain underexplored. How do female sexual behaviors evolve, and how do new behaviors integrate into existing social interactions? Here, we report a newly identified and recently originated female sexual behavior in the *melanogaster* subgroup species *D. santomea*, where females spread their wings in response to male courtship songs. This behavior is observed in multiple strains of *D. santomea* but not in other *melanogaster* subgroup species, thus placing its origination at 0.4 MYA. Wing spreading (WS) is strongly correlated with a female's receptivity state, and WS probability increases before copulation. By abolishing WS in *D. santomea* and closely related species, we show that WS acts as a species-specific signal during courtship to enhance copulation success. This effect is mediated by WS promoting longer male courtship songs, and pairs that engage in this social feedback loop are more likely to copulate. Vaginal plate opening (VPO) is another female receptive behavior and manifests as abdomen extension. We show that WS co-occurs and quantitatively scales with more intense expressions of VPO, suggesting that they share social contexts but convey non-identical information. Lastly, informed by previous reports of WS as a copulation acceptance signal in the *Drosophila* subgenus, we sampled species beyond the *melanogaster* subgroup to understand WS's evolutionary history. WS has evolved multiple times along the *Drosophila* lineage and always co-occurs with VPO and responds to male cues. In summary, we report a newly originated female sexual behavior and describe how it was integrated into the dynamic social interaction during courtship. WS lends itself as a tractable neural comparative model, and parallel efforts in the lab have begun to uncover the underlying neural circuit mechanism. We highlight the value of understanding species-specific female behaviors in developing a comprehensive framework of social behavior evolution, and provide a comparative paradigm for studying the neural mechanism and evolution of newly originated social behaviors.

749T **Adaptive Evolutionary Responses to Heavy Metal Toxicity in Organisms** Geetanjali Sageena Human Resource Development, Indian Council of Medical Research

The widespread dispersion of heavy metals, owing to their extensive application in industrial, residential, agricultural, medical, and technological domains, has prompted inquiries into their potential ramifications on human health and the environment. The deleterious effects of these metallic elements on various organs are well-documented, even at relatively low exposure levels, and they are recognized as systemic toxic substances. The extent of their harmfulness is influenced by the dosage, mode of exposure, chemical composition, as well as the age, gender, genetic makeup, and nutritional status of the affected individuals.

Under such environmental stressors, there can be a notable decline in the overall fitness of populations, exerting a pivotal influence on the evolution and adaptive processes of living organisms. Biological systems have developed diverse compensatory mechanisms to counteract stress, necessitating energy for all cellular functions. Exposure to such conditions can induce significant selective pressure, potentially resulting in phenotypic or genotypic variations. These adaptations play a critical role in comprehending the mechanisms of evolution. Consequently, a solitary environmental stressor can manifest differently across different life stages, with its associated adaptations exhibiting specificity.

In reaction to these challenging conditions, a variety of organism taxa, ranging from insects to invertebrates, have evolved physiological mechanisms to enhance the efficient utilization of energy. To address this fundamental question, I conducted studies on the tolerance of oxidative stress, employing two distinct populations of Drosophila melanogaster. One population exhibited diminished internal energy reserves, yet displayed increased longevity without compromising on reproductive capacity. The response to chemical stimuli at different life stages was evaluated in both populations of D. melanogaster. The primary objective was to unravel the interrelations and trade-offs among life history traits, whether they are rigid or transitory in nature.

750T **Duplicative transposition of the male fertility gene *kl-2* to the Y chromosome triggers gene subfunctionalization in *Drosophila*** Eduardo Dupim[1], Antonio Bernardo de Carvalho[2], Rodrigo Cogni[3], Maria D Vibranovski[1] [1]Departamento de Genética e Biologia Evolutiva, Universidade de São Paulo, [2]Departamento de Genética, Universidade Federal do Rio de Janeiro, [3]Departamento de Ecologia, Universidade de São Paulo

Unique to males, Y chromosomes are considered a potential source for the emergence and specialization of new sex-specific genes. Although there are reports of duplicative transposition of genes from the autosome to the Y chromosome in the well-studied *Drosophila* fruit flies, little is known about the underlying evolutionary mechanisms maintaining both copies. Here, we explore the origin and duplication of the *kl-2* gene: a male fertility factor encoding a dynein heavy chain protein (DHC) of ~4450 aa, associated with sperm flagellar motility in *Drosophila*. Originally located on an autosome, as observed in other Diptera, *kl-2* was transposed to the Y chromosome in the common ancestor of the Drosophilinae subfamily, leaving an autosomal copy in its ancestral site, called *CG9068*, conserved in all analyzed species genomes. While the Y-linked *kl-2* copy keeps the same size and structure, *CG9068* experienced size reduction, encoding a truncated protein with ~1200 aa, retaining only the first functional domain of the ancestral DHC. In contrast to *kl-2*, predominantly expressed in testes, our differential gene expression analysis, based on public RNA-Seq libraries for *D. melanogaster*, reveals that *CG9068* exhibits specific expression in the second antennal segment, housing chordotonal neurons. These auditory sensory cells possess ciliary motility, suggesting that the truncated gene may play a similar role to *kl-2* in spermatozoa. Comparative analysis of outgroups, including the mosquito *Aedes aegypti*, the screw-worm fly *Cochliomyia hominivorax*, and the drosophilid *Rhinoleucophenga americana* (subfamily Steganinae), indicates that the autosomal nonduplicated gene is expressed in both tissues. Moreover, the antenna, exhibits alternative splicing, resulting in the coding of only the first protein domain, mirroring *Drosophila*'s *CG9068*. These results strongly suggest that the ancestral gene was originally expressed in both hearing cells and sperm, with each copy subfunctionalized following the duplication to the Y chromosome, specializing in one cell type. Intriguingly, we detected a vestigial — but significant — expression of the Y-linked *kl-2* gene in male *Drosophila*'s antenna, pointing to a potential conflict with *CG9068*, as both genes appear to interact with the same proteins in the axonemal complex. This system is an illustrative example of how sexual chromosomes promote diversification and the rise of new genes.

751T **A cryptic evolutionary arms race with an unsuppressed selfish chromosome in *Drosophila*** Jackson Bladen, Hyuck-jin Nam, Nitin Phadnis University of Utah

Sex Ratio (*SR*) chromosomes are selfish *X*-chromosomes that selectively eliminate *Y*-chromosome-bearing sperm in the male germline. By manipulating gametogenesis, *SR* chromosomes dominate the viable gamete pool, bias the sex ratio of progeny, and gain an evolutionary advantage – often at the organism's expense. Unchecked, these selfish genetic elements are predicted to spread rapidly and drive populations extinct. Alternatively, they provide strong selective pressure for the rapid evolution of suppressors, which is then predicted to form a cryptic drive system. *SR* chromosomes fueling evolutionary arms races between X-linked distorters, Y-linked targets, and autosomal suppressors is a central idea in evolutionary theory. Theory shows that an unsuppressed state of a distorting chromosome is expected to be observed only transiently. Several well-studied Sex Ratio systems show no evidence of suppression despite the ancient origins of their *SR* chromosomes. This violation of prediction from our theoretical and experimental understanding of evolutionary conflict is known as the "ancient gene drive paradox."

The *SR* chromosome of *Drosophila pseudoobscura* originated nearly one million years ago, shows near-perfect drive in the male germline, and imposes substantial fitness effects on its carrier. In North American populations, the *SR* chromosome can be found at high, stable frequencies. Despite decades of comprehensive surveys, there are no known drive-resistant *Y*-chromosomes or autosomal suppressors for this ancient chromosome. The absence of suppressors of the *D. pseudoobscura Sex-Ratio* chromosome exemplifies the "ancient gene drive paradox" and has remained an unresolved contradiction in evolutionary genetics.

Here, we experimentally test the intragenomic arms race hypothesis by surveying for cryptic suppressors of the ancient, unsuppressed *SR* chromosome in *Drosophila pseudoobscura.* We generated recombinant chromosomes that reflect the ancestral state of the *SR* chromosome in North American populations. In segregation assays using these recombinants, we discovered a partially dominant suppression system against the ancestral distorter. In addition, we discover an *X*-linked locus that is a suppressor-of-suppressor, producing near-perfect distortion even in the presence of autosomal suppressors. We deconstruct the *D. pseudoobscura SR* system as the ancestral distorter, suppressor-of-distortion, and suppressor-of-suppressor consistent with the framework of an ancient, ongoing evolutionary arms race. Further, these results indicate that the contemporary absence of *SR* suppression is merely a transient phase in an ongoing evolutionary arms race. This ancestral drive-suppression system suggests a resolution to the "ancient gene drive paradox."

752T **Divergence in thermal sensitivity of meiosis in related cold tolerant and thermotolerant species** Jessica McNeill, Nathan Brandt, Caiti Smukowski Heil North Carolina State University

Meiosis is a well conserved process required for the formation of gametes in all sexually reproducing species. However, meiotic structures are sensitive to a variety of external factors, which can impact chromosome pairing, recombination, and fertility. For example, the optimal temperature for successful meiosis varies between species of plants, animals, and fungi. This suggests that meiosis is temperature sensitive, and that selection may act on variation in meiotic success as organisms

adapt to different environmental conditions. One lens through which we can better understand the link between temperature and the evolution of meiosis is to investigate how temperature alters meiotic phenotypes within and between related species with different thermal niches. The budding yeast Saccharomyces are an ideal system to investigate this question, as optimal growth temperature is a defining characteristic delineating species of the genus. We thus surveyed three metrics of meiosis in the thermotolerant species *Saccharomyces cerevisiae* and its relative cryotolerant *Saccharomyces uvarum*: the proportion of diploid cells that complete meiosis (sporulation efficiency), the proportion of spores that are viable following meiosis (spore viability), and recombination rate at a range of temperatures from 4°C-42°C. Our results indicate that there are genetic background and temperature effects on recombination rate variation and sporulation efficiency within and between *S. uvarum* and *S. cerevisiae*. In particular, we demonstrate meiotic failure of cryotolerant *S. uvarum* at 30°C, whereas some *S. cerevisiae* strains can complete meiosis at 42°C, indicating a divergence in thermal sensitivity of meiotic processes over the 20 my of divergence between these species. We then conducted mating trials between strains of *S. uvarum* at three temperatures, and demonstrate that high temperatures significantly reduce viable matings between strains. Together, these results suggest that meiotic thermal sensitivity evolves between closely related species and may even result in temporal reproductive isolation if populations/species differ in temperature tolerance.

753T **Investigating Murine Y Chromosome Diversity and its Functional Consequences** Alexa C Michaels[1,2], Beth Dumont[2,3] [1]Jackson Lab, [2]Tufts University, [3]The Jackson Lab

More than 40% of cases of male factor infertility are caused by Y chromosome aberrations, leading to problems such as azoospermia or low sperm counts. Despite its central importance for male fertility, the Y chromosome exhibits extreme structural variability between species and within populations. Recent advances in sequencing technologies are refining our understanding of Y chromosome diversity and divergence, but our understanding of the functional consequences of this variation is lacking. To address this knowledge gap, I am developing moderate-throughput genomic pipelines using state-of-the-art long-read sequencing methods to systematically catalog structural and sequence-level diversity across the Y chromosome of diverse house mouse strains. I have used these pipelines to generate quality assemblies of multiple mouse lines in the past with high enough coverage to use in downstream analysis of structural variation. Additionally, I will link Y chromosome genetic variation with quantitative metrics of male fertility in large panels of genetically diverse mice to assess the functional impacts of Y chromosome diversity for male fitness. My work is predicated on the hypothesis that discoveries from investigations in mice will provide translational insights for human Y chromosome evolution, diversity, and function, even despite poor conservation of sequence and gene-level content of the mouse and human Y. Indeed, multiple features of the Y chromosome are conserved between human and mouse genomes, including PARs, ampliconic genes, and single copy genes with divergent-X-linked homologs. Overall, my work is expected to generate new Y chromosome genomic resources for the mouse, catalog Y chromosome diversity in diverse mouse populations, assess the functional consequences of Y chromosome variation on male fertility, and document mutational mechanisms of Y chromosome evolution.

754T **Karyotype Analysis and Quantification of Male Fertility Metrics in a Novel Wild-Derived Inbred Mouse Strain Panel** Hilda Opoku Frempong[1], Beth Dumont[2], Lydia Wooldridge[2] [1]The Jackson Laboratory and University of Maine, [2]The jackson Laboratory

Wild mice harbor much greater genomic diversity than their laboratory counterparts and present an opportunity to bring increased genomic complexity into the fold of biomedical research. To this end, we have been pursuing a course of phenotypic and genomic investigations on a new panel of wild-derived inbred strains developed from wild-caught house mice from North and South America (The Nachman Panel). Our ultimate goal is to develop a novel high diversity mouse multiparent mapping population founded from a subset of strains in the Nachman Panel. To ensure that crosses between strains do not unmask of genetic incompatibilities that lead to breeding challenges, we pursued a series of karyotype analyses and fertility phenotyping. First, we sought to karyotype strains in this panel to assure the absence of large-scale structural rearrangements. Indeed, Robertsonian translocations are common in wild mouse populations and, if present in these wild-derived inbred strains, could lead to reduced breeding performance and infertility in experimental crosses. We used DAPI-banding of metaphase cell spreads prepared from tail-tip fibroblasts and cytological analyses of meiotic chromosome axes in spermatocyte cell spreads to confirm that all tested strains possess karyotypes with the expected 40 acrocentric chromosomes. Second, we quantified a number of male fertility metrics, including testis weight, sperm density, and sperm morphology in inbred strains of the Nachman Panel and a subset of their derivative F1 hybrids. Overall, F1 hybrids exhibit fertility metrics that exceed those quantified in the inbred parental lines, and even exceed values observed in classical inbred strains. Overall, our findings point to a conserved karyotype in wild mice from North and South America and reveal hybrid vigor in male reproductive parameters. We conclude that genomic incompatibilities are unlikely to impede future breeding efforts involving strains from the Nachman Panel.

755T **Effects of the Breeding Sex Ratio on Genomic Patterns of Variation** William J Spurley, Bret A Payseur Laboratory of

Genetics, University of Wisconsin-Madison

Population genetic inference routinely assumes that equal numbers of females and males contribute to the next generation. Evidence for sex differences in reproductive success and other life history variables indicates that natural populations often violate this assumption. When the breeding sex ratio is biased away from 1:1, the effective population size is reduced, but this reduction is not distributed equally throughout the genome. Shifts in effective population size caused by skews in the breeding sex ratio differ between sex chromosomes and autosomes because of sex chromosomal imbalances between females and males. To investigate the consequences of changes in the breeding sex ratio on genomic patterns of sequence diversity, we conducted coalescent simulations across a range of demographic histories. We show that the breeding sex ratio interacts with demography to differentially shape sequence variation on the X Chromosome and the autosomes. In this context, the breeding sex ratio exerts disparate effects on levels of variation, the site frequency spectrum of polymorphisms, and linkage disequilibrium. We discuss the implications of our results for interpreting comparisons between X Chromosomal and autosomal diversity and we pinpoint combinations of summary statistics that are promising targets for inferring the breeding sex ratio from population genomic data.

756T **Gene-by-environment interactions, gene expression, and body size in house mice from the Americas** Katya Mack[1], Nico Landino[2], Tiffany Longo[2], Sebastian Vera[2], Lilia Crew[2], Kristi McDonald[2], Megan Phifer-Rixey[3] [1]University of Kansas, [2]Monmouth University, [3]Drexel University

Body size in house mice (*Mus musculus domesticus*) covaries with latitude, consistent with Bergmann's Rule. There is clear evidence that genetic variation contributes to this pattern in the Americas. However, traits related to body size are complex not only because they are polygenic, but also because they are sensitive to the environment and, in particular, diet. New wild-derived strains from regions of the Americas have been developed which vary consistently in aspects of body size. In this study, mice from the new strains were fed either a typical breeder or a high fat diet. Our goal was to use diet as a tool to determine whether there was genetic variation for plasticity among the strains, to characterize gene regulatory divergence between strains, and to connect regulatory variants to adaptive variation in body size. Not only was there evidence that diet affected body size, but also that strains differed in phenotypic response to the high fat diet. Gene expression divergence was extensive both among strains and across diet treatments. Finally, using crosses between strains and allele specific patterns of gene expression, we found that *cis*-by-diet interactions are common (and associated with genes involved in metabolism), and that *cis*-regulatory changes are associated with signals of selection from latitudinal transects of wild populations. Together, these results add to our understanding of the genetic basis of Bergmann's Rule in house mice.

757T **Parent-of-origin disruption of growth and metabolism in hybrid mice** Emily Moore[1,2], Fernando Rodriguez-Caro[2], Jessica C Powell[2], Zac Cheviron[2], Cole Wolf[2,3], Jeffrey Good[2] [1]University of Denver, [2]Biological Sciences, University of Montana, [3]Biology, University of Wisconsin

Pregnancy in placental mammals requires a delicate balance of growth and nutrient allocation on the part of mother and developing offspring, with the needs of the two seemingly at odds. One way intimate interactions between maternal and fetal genomes are mediated is through the expression of imprinted genes with parent-of-origin-biased expression. In theory, genomic imprinting could evolve to reduce antagonistic interactions (conflict) or promote cooperation (coadaptation) between the maternal parent and developing offspring. Regardless, both scenarios can generate coevolutionary dynamics that lead to rapid divergence between species. Consistent with this, mammalian hybrids often show parent-of-origin deficits in growth and development indicating that imprinted gene networks are sensitive to hybrid mismatch. However, it is unclear if and how often such hybrid incompatibilities lead to altered metabolism and growth, if there is disruption to gene networks associated with nutrient allocation in development, or if disrupted fetal development may contribute to adult metabolic phenotypes. In this study, we identified patterns of developmental asymmetry, placental gene expression, and adult growth and fat deposition at scaled levels of divergence using reciprocal crosses within and between three lineages of mice: *Mus musculus musculus (mus)*, *M. m. domesticus (dom)*, and *M. spretus (spret)*. By examining gene expression in functionally distinct regions of the placenta, we found enriched paternal expression in placental tissue responsible for nutrient acquisition, and enriched maternal expression in the tissue that coordinates materno-fetal physiology. Asymmetry in adult sizes in reciprocal crosses was found both in hybrids between more closely-related mice (*mus* and *dom*) and more distantly-related mice (*mus* and *spret*), and adult overgrowth is decoupled from placental size in *mus* and *dom* hybrids. To determine whether adult overgrowth was associated with changes in adiposity, we quantified body fat with QMR and collected gonadal white and intrascapular brown fat depots. Overgrown hybrid mice were longer and fatter than reciprocal hybrids and parents. With connections between placental size, and fetal birth weight and adult phenotypes, hybrid mice are a promising model with which to investigate epigenetic controls linking early development with metabolic disease, as well as serving as a model for mechanisms underlying overgrowth in mammalian hybrids.

758T **The Influence of *MUTYH* on Germline and Somatic Mutagenesis Across Species** Candice L Young[1], David Mas-Ponte[1], Annabel Beichman[1], David Ashbrook[2], Suheeta Roy[2], Shelby Hemker[3], Jeanne Fredrickson[4], Brendan Kohrn[4], Ming Yu[5], William M Grady[5], Jacob Kitzman[3], Brian Shirts[4], Robert W Williams[2], Rosana Risques[4], Kelley Harris[1] [1]Genome Sciences, University of Washington School of Medicine, [2]Genetics, Genomics & Informatics, University of Tennessee Health Science Center, [3]Human Genetics, University of Michigan Medical School, [4]Laboratory Medicine & Pathology, University of Washington School of Medicine, [5]Translational Science and Therapeutics Division, Fred Hutchinson Cancer Research Center

DNA repair mechanisms safeguard our genome, with breakdowns in these processes increasing cancer susceptibility. The tissue-specific impact of mutations in repair genes and their role in aging and health are not fully understood. In addition, even modest increases in germline mutation rates can influence congenital disease risk and the rate of evolution. The *MUTYH* gene, which is associated with human cancers and is also a germline mutator in mice, plays a crucial role in preventing C>A transversion mutations by correcting DNA mispairings. Our research focuses on how *MUTYH* variants modulate mutation frequencies in the somatic and germline cells of both humans and mice, shedding light on how repair genes can impact mammalian aging and the evolution of mutagenesis.

In the BXD recombinant inbred mouse strains, certain *Mutyh* alleles have been found to increase the germline C>A mutation rate. To probe the effects of the *Mutyh* sequence variation on somatic mutagenesis in the same mice, we have sequenced whole genomes at 50X coverage from the spleens of aged BXD mice fed low fat and high fat diets. Using Strelka for mutation calling, we are measuring the accrual of high-frequency somatic mutations attributable to age-related clonal hematopoiesis, a phenomenon previously observed in mice but little studied beyond the C57BL/6J strain. With this, we aim to analyze the BXD mutation spectra to investigate how the *Mutyh* genotype, other potential mutator QTLs, and diet can alter the rate and patterns of somatic mutagenesis in the murine bloodstream.

In parallel to studying the impact of murine germline mutators on somatic mutagenesis, we have been testing human somatic mutator alleles for germline mutator activity. To achieve this, we quantify *MUTYH*'s impact on *de novo* mutagenesis in a 3-generation human family pedigree. By comparing mutations observed in the children of parents with and without pathogenic *MUTYH* mutations, we highlight an association between pathogenic *MUTYH* variants and increased rates of heritable C>A *de novo* mutations. We identify this rate increase in children of mothers but not fathers harboring biallelic pathogenic *MUTYH* mutations, suggesting that the oxidative stress being repaired by *MUTYH* has a proportionally larger effect on the female germline. This study broadly advances our understanding of the role of *MUTYH* and oxidative stress in cancer susceptibility and genomic evolution, and sex-specific reproductive aging in humans.

759T **Investigating the drivers of interspecific variation in antibody immunity using comparative genomic and phylogenetic approaches** Yana Safonova[1], Matt Pennell[2], Anton Bankevich[1], Corey Watson[3] [1]Penn State University, [2]University of Southern California, [3]University of Louisville

A central challenge faced by all organisms is how to defend themselves against pathogens and parasites, particularly those that evolve rapidly. Across the Tree of Life, evolution has devised many ingenious solutions to this problem. Arguably, the most elegant of these is the adaptive immune system of vertebrates that includes antibody immunity and T-cell immunity and in which evolution stumbled upon the idea of using evolution itself to combat a wide variety of infections. The fundamental principles of adaptive immunity are conserved among vertebrates, suggesting a single evolutionary origin. However, the relatively limited set of studies that have investigated adaptive immunity across species have uncovered remarkable variation in adaptive immune repertoires, as well as in the genetics underlying them. A fundamental question is: what are the evolutionary causes and consequences of this variation? Comparative studies of the adaptive immune systems have been infeasible until now owing to technical limitations that have only recently been overcome.

In this work, we conducted the first phylogenetic comparative study of adaptive immune systems with the focus on antibody immunity. We developed novel computational tools for annotation of germline immunoglobulin (IG) loci and expressed antibody repertoires encoded by them and applied these tools for a wide range of mammalian species. We also used comparative phylogenetic tools to reveal the evolutionary history of IG genes encoding antibodies.

As a result, we revealed numerous species-specific adaptations of adaptive immune systems including non-canonical antibodies with therapeutics potential. We also described rapidly evolving regions of immunoglobulin loci and showed that they are responsible for generation of new IG genes. Finally, we formulated hypotheses linking the genetic diversity of IG loci with the diversity of expressed antibody repertoires.

760T **HIV rapid intra-host evolution allows evasion from VRC01 infusion via positive selection** Frida Belinky[1], Sung Hee Ko[1], Pierce Radecki[1], Vanessa Guerra Canedo[1], Emily Elizabeth Coates[1], Pamela J M Costner[2], Julie E Ledgerwood[1,3], John R Mascola[1,4], Eli A Boritz[1] [1]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, [2]National Institute of

Allergy and Infectious Diseases, [3]CONSORTIUM FOR HIV/AIDS VACCINE DEVELOPMENT, [4]ModeX Therapeutics Inc

One of the factors contributing to HIV's persistence is its rapid evolution. Understanding how the *env* gene evolves under antibody-mediated pressure is particularly important for efforts to use antibodies as therapies for HIV. In a previous clinical trial, infusion of the broadly-neutralizing antibody (bNAb) VRC01 lowered plasma HIV viremia in a subset of participants, but was associated with the emergence of neutralization-resistant *env* variants. Here we sought to develop an analytical approach for identifying *env* mutations that confer escape from antibodies using single-genome sequence data from participants treated with VRC01. We applied single-genome amplification and sequencing (HT-SGS) using unique molecular identifiers (UMIs) and the Pacific Biosciences long-read platform, to measure HIV *env* allele frequency changes over time in longitudinal samples from people with chronic, untreated HIV infection who received one dose of VRC01, which targets the CD4 receptor binding site region of HIV *env*. Eight participants were studied, and a total of 29,433 sequences were analyzed. We used two approaches to identify changes adaptive to VRC01 escape. The first was a codon-based dN/dS approach executed by HYPHY with the FUBAR algorithm to identify positions under positive selection at only the post-infusion timepoint. The second approach is a population genetics approach where positive selective sweeps along the *env* gene are identified with Tajima's D and other measures, and then a score for the Selected Allele Favored in Evolution (SAFE) is calculated for each variation with >0.1 frequency change between pre and post infusion. A panmixia analysis revealed that in six of eight individuals the viral populations post infusion were distinct from the pre infusion populations. Specifically, the VRC01 epitope is changed in these six individuals, as seen by logo plots comparison between pre and post infusion. In two individuals, both FUBAR and SAFE approaches identified the same positions as adaptive. In another four individuals FUBAR and SAFE pointed at different residues as adaptive. Examining manually regions of selective sweeps revealed complex evolutionary scenarios and suggest the existence of several adaptive mutations in each host, with possible epistasis between positions as suggested by the existence of low frequency combinations of the adaptive mutations that do not grow in frequency. In two individuals, subpopulation structure correlating with sequence signatures of cell tropism is associated with different escape mutations. These results suggest diverse virological responses and adaptive pathways to bNAb escape among different individuals. While some mutations are easily identified as adaptive, the existence of multiple escape mutations in a single host, as well as sub populations may result in more obscured signals that are more challenging to interpret.

761T **How mammals lost their vision, according to genetics** Sarah Lucas[1], Charles Feigin[2], Stephen Frankenberg[2], Andrew Pask[2], Nathan Clark[3] [1]Human Genetics, University of Utah, [2]School of BioSciences, University of Melbourne, [3]Biological Sciences, University of Pittsburgh

Vision is a critical sensory system for survival in many mammalian species. It is therefore fascinating that a trait so critical to many mammals has become dispensable and repeatedly lost in several independent lineages including moles, echolocating bats, shrews, armadillos, and several others. While possible, it is unlikely that these species instantaneously became blind. Instead, they likely underwent a process to lose their vision over millions of years. What remains unclear is if they all went through the same process to lose their vision. Most vision-loss research has focused on documenting changes in genes vital for rod and cone photoreceptor function within the retina. While these cell types convert dim and colored light respectively into signals that the brain can detect, they are not the only cell types important for vision. One understudied cell type is the lens fiber cells which ensures a clear image is focused onto the photoreceptors. To understand if there is a universal process whereby mammals undergo vision-loss, we compared the change in constraint of three cell types: lens fiber cells, cone photoreceptors, and rod photoreceptors across poorly sighted species and their sighted sister taxa. We measured the constraint of a given cell type by collectively assessing cell-type specific gene groups and compared the observed codon changes between the orthologs of the poorly sighted species and its sister taxa. Our study revealed that the most frequent cell type to lose constraint in the poorly sighted species was the lens, followed by rod and then cone photoreceptors. We found multiple independent instances of loss in the Eulipotyphla (moles and hedgehogs) and Chiroptera (bats) clades. Within Xenarthra we discovered armadillos only lost constraint of their rods while sloths lost constraint only of their cones. Many species lost constraint in multiple cell types and in quick succession leaving it difficult to determine an ordering; however, in the cases where we have enough power to detect a difference, the constraint was lost first in either the lens or cones before rods. This suggests that constraint among these related cell types is independent and hints that there could be a preferred process towards vision loss within mammals. This study revealed how a sensory system is lost by comparing how changes in constraint affect individual components of this complex system across a broad and diverse mammalian phylogeny.

762T **A polygenic explanation for Haldane's Rule in butterflies** Tianzhu Xiong[1], Shreeharsha Tarikere[2], Neil Rosser[2], Xueyan Li[3], Masaya Yago[4], James Mallet[5] [1]Molecular Biology and Genetics, Cornell University, [2]Organismic and Evolutionary Biology, Harvard University, [3]Kunming Institute of Zoology, Chinese Academy of Sciences, [4]The University Museum, The University of Tokyo, [5]Harvard University

Two robust rules have been discovered about animal hybrids: heterogametic hybrids are more unfit (Haldane's Rule), and sex

chromosomes are disproportionately involved in hybrid incompatibility (the large-X/Z effect). Exact mechanisms causing these rules in female heterogametic taxa such as butterflies are unknown, but are suggested by theory to involve dominance on the sex chromosome. We investigate hybrid incompatibilities adhering to both rules in *Papilio* and *Heliconius* butterflies, and show that dominance theory cannot explain our data. Instead, many defects coincide with unbalanced multilocus introgression between the Z chromosome and all autosomes. Our polygenic explanation predicts both rules because the imbalance is likely greater in heterogametic females, and the proportion of introgressed ancestry is more variable on the Z chromosome. We also show that mapping traits polygenic on a single chromosome in backcrosses can generate spurious large-effect QTLs. This mirage is caused by statistical linkage among polygenes that inflates estimated effect sizes. By controlling for statistical linkage, most incompatibility QTLs in our hybrid crosses are consistent with a polygenic basis. Since the two genera are very distantly related, polygenic hybrid incompatibilities are likely common in butterflies.

763T **Innovation, constraint, and the evolution of genetic networks in major eukaryotic lineages** Jacob Steenwyk[1], Maxwell C. Coyle[1], Noah Bradley[2], Xiaofan Zhou[3], Yuanning Li[4], Xing-Xing Shen[5], Chris Hittinger[6], Antonis Rokas[7], Nicole King[1] [1]UC-Berkeley / HHMI, [2]Northwestern University, [3]South China Agricultural University, [4]Shandong University, [5]Zhejiang Univeristy, [6]University of Wisconsin - Madison, [7]Vanderbilt University

Genetic networks depict the intricate relationships among genes, their pathways, and cellular functions. Here, we infer genetic networks using coevolutionary information across 26 major lineages of animals and fungi. Analysis of network features uncovers both analytical and biological factors influencing their structural properties, including evolutionary rate and signal-to-noise ratios. Ancestral reconstructions of complex gene-gene relationships uncover patterns of gain and loss, mirroring gene families, but substantially more dynamic. Guided by these findings, we construct individual genetic networks for Animals and Choanoflagellates and identify complex gene relationships shared in both lineages, thus likely predating animal origins. Shared hubs of genes encode ancient cellular functions, such as cell cycle regulation, DNA replication, and ciliary processes. The principle of 'guilt-by-association' emerges as a promising approach for uncovering genotype-to-phenotype relationships. These findings uncover innovation and constraint in genetic network evolution and suggest that gene-gene association changes are a dynamic and underexplored mode of genome evolution.

764T **Large genotype frequency fluctuations are linked to decorrelated offspring number stochasticity** Joao A Ascensao[1], Kristen Lok[2], Oskar Hallatschek[1] [1]University of California, Berkeley, [2]Duke University

Understanding the factors that drive population fluctuations is crucial for predicting and controlling ecological and evolutionary dynamics. In this context, the role of strain interactions and their impact on population fluctuations has remained largely unexplored. Our group and others have observed large population fluctuations that are difficult to explain through genetic drift, thought to be the primary source of genotype frequency fluctuations. We developed a model to delve deeper into this issue, focusing on the dynamics of genotypes within a community. Genetic drift arises from independent offspring number fluctuations. However, when offspring fluctuations are correlated between individuals, fluctuations appear with distinctive scaling behaviors. The correlated offspring fluctuations can strongly affect the total population abundance, and can also affect the genotype frequency if the correlated fluctuations between genotypes are sufficiently decoupled from each other. We thus refer to these frequency fluctuations as "decoupling fluctuations". Empirical measurements using model microbial populations quantitatively supported our model, revealing that decoupling fluctuations between strains can lead to frequency fluctuations far larger than those from genetic drift. We also explored the evolutionary implications of these decoupling fluctuations, showing that they can significantly influence the fixation probability of a strain and the site frequency spectrum. Notably, decoupling fluctuations arise from a sufficiently generic mechanism such that we expect they are common across biological populations, strongly affecting evolutionary dynamics. Overall, our findings highlight the importance of considering correlated fluctuations in understanding population dynamics and their evolutionary consequences.

765T **Recombination mitigates selection against Neanderthal introgression in shaping introgression landscape** David Y. Lu, Ziqing Pan, April Wei Computational Biology, Cornell University

Genome analyses have shown that present-day humans of non-African descent acquire 1-4% of their genetic material from Neanderthals as a result of human-Neanderthal admixture around 50,000 years ago. Previous studies suggest the initial fraction of Neanderthal introgression could be as high as 10% based on forward-time simulations with human demographic history and distribution of fitness effects (DFE). However, these simulations all adopt a uniform recombination rate, despite the fact that variation in recombination rate is expected to shape introgression patterns. The 10% initial introgression proportion is also unrealistic given the small effective population size of Neanderthals. Here, we examine selection against Neanderthal introgression by building a more realistic forward-time simulation framework utilizing annotated exonic regions from the UCSC Genome Browser, distribution of mutational fitness effects, and fine-scale recombination rates from deCODE genetics. Employing tree sequence encoding, we efficiently simulate the 2.9 Gbps whole-genome for 62,000 generations

and compute Neanderthal ancestry proportion across the human genome at 25 time points since introgression. We show that under mutation models that assume recessive, partial recessive, and additive fitness effects, an admixture fraction of around 5% instead of 10% would better explain the observed Neanderthal ancestry in present day humans. We find Neanderthal ancestry is enriched in high recombination rate regions. Furthermore, the purging of Neanderthal ancestry after introgression is influenced by both local recombination rate and fitness of the Neanderthal segment. Our simulation also better explains protein coding and non-coding specific patterns of Neanderthal ancestry, patterns of introgression deserts, and the relationships between gene density, recombination rate, and Neanderthal ancestry proportion. In conclusion, initial Neanderthal introgression could be much lower than previously suggested. Our results reiterate the importance of the interplay between recombination and purifying selection. A high resolution, realistic recombination map and gene annotation map are essential to understanding both genome-wide and localized patterns of introgression. It is particularly important to incorporate realistic maps in studies of genetic rescue, where variable recombination rates along the genome have not yet been utilized.

766T **Evaluating cluster-based transmissibility measures under a phylodynamic framework with the structured coalescent** Peiyu (Perry) Xu, Shenni Liang, Andrew G Clark, Jaehee Kim Cornell University

In the genetic epidemiology of infectious diseases, characterizing transmissibility and detecting high-transmissibility variants based on the genealogy of the pathogen is a major topic of interest. Current popular measures for transmissibility include the clustering rate (CLR), the size of clusters (CLS), and the length of terminal branches (TBL). These measures are largely based on clusters, which are defined as subtrees in which all descendant tips have a maximum patristic distance below a prespecified threshold, reflecting a group of recent transmission events. However, CLR, CLS, and TBL lack a rigorous theoretical foundation and are primarily based on empirical observations.

Bridging this gap, phylodynamic models offer a more rigorous approach by harnessing information from the sequences of disease agents, which allows for a deeper understanding of the evolutionary and epidemic dynamics of pathogens. The framework operates under the assumption that a genealogy represents a partially observed undirected transmission tree, with the sampled hosts represented by leaves and unobserved transmission events represented as internal nodes. Probabilistic models of genealogies, coalescent or birth-death-sampling (BDS) models, are employed to link observed genetic relationships among pathogens to unobserved epidemiological relationships between infected individuals.

Here, we leverage the phylodynamic framework with a structured coalescent model to 1) derive analytical expressions for CLR, CLS, and TBL under SIR and SEIR models; 2) examine their associations with transmissibility and other epidemiological parameters, assessing the validity of CLR, CLS, and TBL as proxies for transmissibility; and 3) validate our analytical results both with synthetic and real whole genome sequencing data of SARS-CoV-2 and *Mycobacterium tuberculosis*. Our work provides a foundation for current and future methods for transmissibility inference and can aid in the development of more accurate and robust computational tools for infectious disease control and prevention guided by phylodynamic principles.

767T **Genetic diversity and population structure of creole goats from northern Peru revealed by genome-wide SNP markers** Flor-Anita Corredor[1], Deyanira Figueroa[1], Richard Estrada[1], William Burgos-Paz[2], Wilian Salazar[1], Wilder Cruz[1], Roiser Lobato[1], Pedro Injante[1], David Godoy[1], Christian Barrantes[1], Juancarlos Cruz[1], Carlos I. Arbizu[1,3] [1]Instituto Nacional de Innovación Agraria - INIA, [2]Corporación Colombiana de Investigación Agropecuaria-AGROSAVIA, [3]Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas

The single nucleotide polymorphisms (SNP) chips for massive genotyping, are useful for the effective management of genetic resources. This research represents the initial effort to assess the genetic variation and population composition of creole goats from northern Peru through SNP markers. This study involved the collection of 192 male creole goats from three key goat production regions in northern Peru. These goat samples were genotyped using the GGPGoat70k SNP panel. To investigate the genetic influence of other breeds on Peruvian creole goats, our dataset was combined with previously published SNP genotypes. Estimations for expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (F_{IS}) were computed for the Peruvian groups. Subsequently, we conducted principal component analysis (PCA), admixture analysis, and AMOVA using the consolidated data. The results revealed a considerable genetic diversity, with He values ranging from 0.41 to 0.42 for the Peruvian sampling groups. The inbreeding coefficient was notably low for Peruvian goat and population structure analysis demonstrated a distinction ($p< 0.05$) from other breeds. These findings suggest a level of differentiation of the Peruvian goat population among other breeds, although further research is needed considering samples from other production areas (work in progress). We expect this study will contribute to defining genetic management strategies to prevent the loss of genetic diversity in Peruvian goat populations and for upcoming advancements in this field.

768T **Utilizing Fractional Calculus to Investigate the Impact of Heterogeneity in Population Genetics** Somayeh Mashayekhi[1], Peter Beerli[2] [1]Mathematics, Kennesaw State University, [2]Florida State University

The Wright-Fisher model and Kingman coalescent model occupy an essential role in studying the evolutionary history of a population. The coalescent process, based on the backward process in time, is dual to the Wright-Fisher model, which describes the evolution of a population forward in time and is a mathematical framework for modeling allele frequency data. Although the dynamics of the Wright-Fisher model are well understood, a simple closed-form analytical expression for the distribution of allele frequencies (DAF) is unknown. The diffusion approximation is a well-known method for calculating the DAF by considering the effect of evolutionary and demographic forces such as genetic drift, mutation, selection, and immigration.

Although Natural populations reside in diverse environments, and individuals within these populations exhibit varying opportunities for reproduction, the existing population genetics models assume homogeneity within a population's environment. In recent developments, we have pioneered the application of fractional calculus in population genetics, introducing the fractional coalescent as an extension of the Kingman coalescent. This model enables the study of population heterogeneity, leveraging the Mittag-Leffler distribution function to model waiting times. The concept of altering the probability distribution of waiting times is rooted in the influence of heterogeneity on the number of offspring. We have demonstrated that when the variance in the number of offspring is not constant and fluctuates, perhaps due to the impact of heterogeneity, then the distribution of waiting times follows a Mittag-Leffler distribution.

Additionally, recognizing that the coalescent model is typically confined to a small number of individuals and does not incorporate allele frequency data, we have introduced a new framework based on the fractional diffusion equation. This framework focuses on the impact of heterogeneity by employing fractional calculus to estimate the distribution of allele frequencies (DAF). To derive the DAF using the diffusion equation, we've developed a computational method based on the well-established spectral method. This presentation will explore the fundamentals of fractional coalescent and fractional diffusion equations in population genetics. We will also provide illustrative examples to showcase the advantages of these methods.

769T **Non-heritable yet evolvable: increased developmental noise can be selected for despite average negative effect** Csenge Petak[1], Thomas Sgouros[2], Lapo Frati[1], Ignacio Bravo[3], Daniel Weinreich[2] [1]University of Vermont, [2]Brown University, [3]Centre National de la Recherche Scientifique

Population genetics typically considers the fate of genetic variants in the population that have direct fitness effects on the carrier. However, the effect of certain genetic variants is that they influence the distribution of phenotypic variation that is generated each generation. Previous studies have investigated the fate of mutations that increase noise during reproduction, resulting in increased mutation rate. In this study, we used a simple two-locus two-allele model to also investigate the fate of mutations that influence noise during development, increasing the range of adult phenotypes given the same genotype. We find that the specific phenotypic variation generated need not be heritable for the ability to generate a wider range of phenotypes to spread through indirect selection in the population. Moreover, an allele increasing developmental noise can reach fixation even when most (but not all) random variation is disadvantageous and thus on average the allele has a negative effect on fitness. Surprisingly, we find that the probability of fixation increases with increasing selection pressure, and decreases with increasing population size. The implications of these results are potentially far reaching. Selection resulting in higher developmental noise in the population could influence the ability of the population to adapt rapidly to sudden changes in the environment, which is essential in many fields of biology including climate change adaptation, antimicrobial and cancer treatment resistance.

770T **The Population Genetics of Biological Noise** Csenge Peták[1], Thomas Sgouros[2], Lapo Frati[1], Yevgeney Raynes[2], Ignacio Bravo[3], Daniel Weinreich[2] [1]University of Vermont, [2]Brown University, [3]CNRS

Each generation, biological information is transmitted from zygote to adult during development, and back to the zygote during reproduction. Both channels -- development and reproduction -- are susceptible to noise, which can have phenotypic consequences. And while noise is an inherent feature of information transmission, in biological systems its magnitude can be under heritable control. For example, DNA polymerase alleles can differ in mutation rate, promoter alleles can differ in the level of noise in gene expression, and ribosomal protein alleles can differ in translational accuracy. Consequently, populations can carry heritable variation in the amount of phenotypic variation they carry, the latter being precisely the substrate for natural selection. This raises the question of how natural selection regards variants that influence the amount of biological noise. While others have explored this issue in individual cases, we present what we believe is a novel, synthetic population genetic framework for understanding this problem in all settings. We argue from first principles that phenotypic noise will on average be deleterious, but can also occasionally give rise to rare, beneficial phenotypes. We find that the fate of noise-regulating alleles thus depends on the statistics of the phenotypic variation they engender and the persistence of their association with said phenotypic variation, as well as classical features of the population such as its size. We illustrate these ideas with specific biological examples as well as introducing several important, open questions.

771T **Modelling Human Population Growth with an Integrated Archeological-Genetic Framework** Gabriel Kennedy, Shyamalika Gopalan, Amy Goldberg Evolutionary Anthropology, Duke University

Inference of human population size over time is important for understanding human evolution, anthropological impacts, and other population genetic questions. Towards this goal, differing methods independently use genomic or archeological data to reconstruct population size over time. From the earliest days of population genetics, theory has linked genetic variation to estimates of effective population size. However, the relationship to census size is more complicated. Interpretation of population size from genomic data is further exacerbated by poor temporal resolution on recent timescales and varying generational times. In parallel, archeological models have used the distribution of radiocarbon dates as a proxy for human census size, under the assumption that greater numbers of people will leave more material behind, with fine-scale spatial and temporal resolution. However, these methods are sensitive to taphonomic and sampling biases and cannot differentiate between endogenous growth versus migration. Here, we present an Approximate Bayesian Computation framework to infer human population size over time using genomic and archeological data simultaneously to leverage their respective strengths. This method combines summary statistics from both types of data, using SLiM for genetic simulations and *rcarbon* for radiocarbon dates to estimate distributions of population growth parameters. Under the model, we can estimate population growth rates and the timing of size changes at high temporal resolution. We compare the information gained by the joint model to inference using each data type independently and consider which summary statistics are informative to create a combination that utilizes the strengths of both data types.

772T **The effect of heterogeneous recombination landscapes on the probability of sex chromosome turnover** Mark Hibbins, Stephen Wright Ecology and Evolutionary Biology, University of Toronto

Rates of sex chromosome turnover vary widely across the tree of life. Current models suggest that the turnover of sex chromosomes may be promoted by degeneration of existing sex chromosome systems and the accumulation of sexually antagonistic variation on autosomes. An overlooked factor in such models is the genomic landscape of recombination, which can interact with these mechanisms. In many organisms, recombination is not uniform across the genome, but is elevated in “hotspots” of high recombination separated by large regions of relatively low recombination. This landscape can additionally vary among the sexes; male recombination is often more biased towards the ends of chromosomes, for example. Pre-existing regions of low recombination may promote the turnover of sex chromosomes by capturing and linking sexually antagonistic variation, a reversal of the traditional framework of sex chromosome evolution in which recombination suppression evolves after the genesis of a sex-determining region. Here, we investigate how the landscape of recombination promotes the turnover of sex chromosomes using a forward simulation study. We allow sexually antagonistic variation to accumulate on simulated autosomes with different landscapes of recombination and track the fate of novel sex-determining loci introduced to these autosomes. Our results provide new insights into the mechanisms promoting sex chromosome turnover among lineages.

773T **Solving the Arizona search problem by imputation** Egor Lappo, Noah A. Rosenberg Biology, Stanford University

In forensic genetics, an “Arizona search” is an evaluation of the numbers of pairs of profiles in a forensic-genetic database that possess partial or complete genotypic matches. An Arizona search assists in establishing the extent to which a set of forensic loci provides unique identifications of individuals, and it also enables a test of the accuracy of mathematical models that predict match probabilities for locus sets. For sets of short-tandem-repeat (STR) loci in use in forensic genetic marker systems, however, the potential for performing Arizona searches is constrained by the limited availability of actual forensic profiles for research purposes. Here, we propose the use of genotype imputation as a method to circumvent this problem. From a database of genomes, we impute genotypes of STR loci based on profiles of neighboring single-nucleotide polymorphisms (SNPs), searching for partial STR matches in the database on the basis of the imputed profiles. We compare the numbers of partial matches in imputed profiles to those of the actual profiles, finding a close agreement in the fractions of pairs that possess various levels of partial match. As the size of publicly available datasets on SNPs and whole genomes continues to grow, the results suggest that despite the limited potential for performing Arizona searches with actual forensic STR profiles, the questions that such searches seek to answer can be posed by conducting Arizona searches using imputation in increasingly large SNP databases.

774T **LD and other between-LOCUS associations under drift, population structure, and tight linkage: A likelihood-based approach** Marcy Uyenoyama Biology, Duke University

Observations of genome- and planet-wide patterns in linkage disequilibrium and other forms of genomic associations have served as the basis of a variety of inferences regarding demographic history. In some applications, such indices are explicitly recognized as indicator phenomena associated with population structure. Clarification of the theoretical basis of the generation of genetic associations would facilitate the placement of such inferences within a likelihood-based framework.

I describe a compact method for deriving likelihoods based on samples of two-locus haplotypes under tight linkage. Of particular interest are associations that arise among sites separated by just tens of kilobases, implying rates of crossing-over on the order of 10^{-5}. Interactions among genetic drift, mutation, crossing-over, and regular inbreeding determine levels of within-locus genetic diversity and the nature of associations between loci. Analogues of classical measures of disequilibrium emerge from these likelihood distributions of identity.

775T **Ancient gene duplication and recent non-coding structural variation underpin pigmentation diversification in swordtail (*Xiphophorus*) fishes** Tristram O Dodge[1], Daniel L Powell[1], John J. Baczenas[1], Theresa R Gunn[1], Manfred Schartl[2,3], Molly Schumer[1,4] [1]Biology, Stanford University, [2]Developmental Biochemistry, University of Wuerzburg, [3]Xiphophorus Genetic Stock Center, Texas State University, [4]Howard Hughes Medical Institute

Pigment pattern variation has long interested biologists as striking examples of phenotypic diversification and is a tractable system to understand molecular genetics and development. While mammalian melanocytes have been most thoroughly studied, teleost fish provide an exciting avenue for pigmentation studies, due to their high levels of pattern variation and repertoire of five pigment cell types. This diversity is hypothesized to have arisen in part from the teleost whole-genome duplication 350 million years ago; however, few pigmentation genes have been identified in fish, making this hypothesis difficult to evaluate. Swordtail fish in the genus *Xiphophorus* are polymorphic for a dizzying array of pigmentation traits under balancing selection, providing unique opportunities to characterize their genetic architecture and evolution.

We leverage genome-wide association studies, long-read sequencing, chromatin accessibility assays, and gene expression data to uncover the genetic architecture of multiple pigmentation patterns in swordtails. We find that structural variants upstream and downstream of ancient *kit-ligand* paralogs underpin multiple distinct and convergent phenotypes across two clades. In one swordtail clade, we pinpoint the genetic basis of three distinct melanic ornaments on the body and tail to different non-coding regions upstream of *kitlga*, a duplicated gene thought to maintain the ancestral pigmentation function of *kit-ligand* in other vertebrates. We use long-read sequencing across populations and species to identify complex structural variation in these regions ranging from 30-100kb that increase tissue-specific expression of *kitlga*, and we profile the chromatin accessibility in these regions. In a second swordtail clade, we find a distinct series of tailspot phenotypes map to structural variation upstream and downstream of *kitlgb*. We show *kitlgb* functions a pigmentation gene in *Xiphophorus*, despite loss of this ability in zebrafish.

These results represent a remarkable case of convergent evolution and phenotypic diversification, where ancient *kit-ligand* paralogs have been recently and recurrently modified to produce an array of phenotypes. More generally, our results show that ancient whole-genome duplications can set the stage for recent phenotypic diversification. Moreover, long-read sequencing can uncover complex structural variants in non-coding regions, which may play an underappreciated role in gene regulation and trait evolution.

776T **Integrating epidemiological and population genetic models of *Plasmodium vivax* genomic variation** Shyamalika Gopalan[1], Jillian Grassia[2], Amy Goldberg[1] [1]Evolutionary Anthropology, Duke University, [2]Duke University

Plasmodium vivax, a causative agent of malaria, is a growing public health concern across Asia, South America, and Eastern Africa. Increasing quantities of genomic data are being generated from infected patients with the goal of improving our understanding of *P. vivax* transmission dynamics and parasite biology. However, interpreting these data to yield concrete insights remains difficult, and will require new models that account for *P. vivax*'s unusual life cycle. This life cycle involves obligate haploid and diploid stages within the human host and mosquito vector, respectively, as well as period of extreme exponential growth and extreme bottlenecks, features that would be expected to produce complex patterns of neutral genetic variation. Here, we present a model of *P. vivax* that captures important aspects of the parasite's biology in order to simulate neutral variation across its entire genome. A key feature of our model is that it connects classic epidemiological models (i.e. Ross-Macdonald style models) with recently developed forward genomic simulation methods to quantify the impact of epidemiological and population genetic parameters on *P. vivax* genetic diversity. In exploring this integrated model, we find that the severity of the human-mosquito transmission bottleneck has a much greater effect on pairwise diversity than does total host parasitemia (i.e. host carrying capacity). This result is consistent with population genetic theory, which predicts that population bottlenecks will have a disproportionately large impact on effective population size and diversity. More generally, by enabling simulations of realistic neutral variation, our work opens up opportunities for investigating many aspects of malaria parasite evolution, including the impact of natural selection on genomic loci that depart significantly from baseline expectations.

777T **Adaption to repeated long-term starvation illustrates how microbes navigate complex stress** Megan G Behringer[1], Wei-Chin Ho[2], Sarah Worthan[1], Robert McCarthy[1] [1]Biological Sciences, Vanderbilt University, [2]Biology, University of Texas at

Tyler

Microbes are evolutionarily robust organisms capable of rapid adaptation to complex stress, which enables colonization of harsh environments. In nature, microbes are regularly challenged by starvation, a particularly complex stress, as resource limitation often co-occurs with changes in pH, osmolarity, and toxin accumulation created by metabolic waste. Additionally, complications introduced by eventual resource replenishment are an often-overlooked challenge encountered by microbes adapting to starvation. Here, successful microbes must withstand rapid environmental shifts before swiftly capitalizing on replenished resources to avoid invasion by competing species.To understand how microbes navigate trade-offs between growth and survival, ultimately thriving in environments despite sporadic resource availability, we experimentally evolved *Escherichia coli* populations for 900 days to repeated cycles of 100-day starvation before resource replenishment. Metagenomic analysis revealed that reduced mutation rates, frequent parallel variants, significant mutational order, and successive selective sweeps are associated with adaptation to repeated long-term starvation. Parallel variants often arose in global gene expression regulators, and 7/16 populations evolved an R109H substitution in the transcriptional terminator Rho. Genetic reconstruction revealed Rho^{R109H} confers pH-sensing activity, contributing to increased fitness upon resource replenishment as environmental pH rapidly decreases. Rho^{R109H} also enables efficient resource scavenging from necromass by improving metabolic capability on exogenously acquired intermediates of central metabolism. We further identified naturally occurring Rho^{H109} residues in microbial species regularly challenged by alkaline to neutral pH shifts. Our results demonstrate that experimental evolution can provide insights into how microbes adapt to extreme environments, highlighting the previously unknown functional diversity of organisms that thrive in these environments.

778T **Using ancient genomes to analyze the history of Neanderthal DNA in Europeans and East Asians** Abigail E Kuntzleman[1], David Rinker[2], Laura Colbran[3], John A Capra[4] [1]Computational Biology, Brown University, [2]Vanderbilt, [3]University of Pennsylvania, [4]University of California San Francisco

All non-African human populations carry approximately 2% DNA of Neanderthal ancestry in their genomes. The growing number of genome sequences from ancient humans provides the opportunity to track trends of Neanderthal DNA frequency, selection, and populations-specificity in antiquity. Here we use 301 newly available ancient genomes from across Eurasia to demonstrate that Neanderthal ancestry in modern humans has remained consistently low over the past 50,000 years; this confirms previous work based on a smaller number of ancient human genomes. We then use evolutionary simulations to show that simple neutral selection admixture models are not sufficient to explain trends of introgressed haplotype frequencies observed in real data. To explore potential functional drivers of selection on introgressed haplotypes, we analyze Neanderthal-introgressed variants previously predicted to be under positive selection in OAS2, UBE2F, TLR1, BNC2, and GORAB using phenotype data from the UK Biobank. These loci are significantly associated with phenotypes related to immune function, hair and skin color, and male pattern baldness. We use ancient genomes to show that these variants have remained at high frequency in Europeans for at least 8,500 years and infer that these genes exhibit differing histories in East Asians. Our results trace trends of Neanderthal ancestry over time and identify population-specificity in some introgressed regions. Overall, we illustrate how ancient genomes can now be analyzed at the population-level, and we highlight the need for more ancient genomes, particularly in non-European populations.

779T **Characterization of the *Pristionchus pacificus* "epigenetic toolkit" reveals the evolutionary loss of the histone methyltransferase complex PRC2** Audrey Brown[1], Adriaan B Meiborg[2], Mirita Franz-Wachtel[3], Boris Macek[3], Spencer Gordon[1], Ofer Rog[1], Cameron J Weadick[4], Michael Werner[1] [1]School of Biological Sciences, University of Utah, [2]Developmental Biology, EMBL, [3]University of Tübingen, [4]Biosciences, University of Exeter

Comparative approaches have revealed both divergent and convergent paths to achieving shared developmental outcomes. Thus, only through assembling multiple case studies can we understand biological principles. Yet, despite appreciating the conservation – or lack thereof – of developmental networks, the conservation of epigenetic mechanisms, which regulate those networks, is poorly understood. The nematode *Pristionchus pacificus* has emerged as a model system of plasticity and epigenetic regulation as it exhibits a bacterivorous or omnivorous morph depending on its environment. Here, we determined the "epigenetic toolkit" available to *P. pacificus* as a resource for future functional work on plasticity, and as a comparison with *C. elegans* to investigate the conservation of epigenetic mechanisms. Broadly, we observed a similar cast of genes with putative epigenetic function between *C. elegans* and *P. pacificus*. However, we also found striking differences. Most notably, the histone methyltransferase complex PRC2 appears to be missing in *P. pacificus.*We describe the deletion/pseudogenization of the PRC2 genes *mes-2* and *mes-6* and conclude that both were lost in the last common ancestor of *P. pacificus* and a related species *P. arcanus.* Interestingly, we observed the enzymatic product of PRC2 (H3K27me3) by mass spectrometry and immunofluorescence , suggesting that a currently unknown methyltransferase has been co-opted for heterochromatin silencing. Altogether, we provide an inventory of epigenetic genes in *P. pacificus* to enable reverse-genetic experiments related to plasticity, and in doing so describe the first loss of PRC2 in a multicellular organism.

780T **Population genomics of unusually large white-footed mice in the Boston Harbor archipelago** Emma Howell[1], Lauren Nolfo-Clements[2], Bret Payseur[1] [1]Genetics, University of Wisconsin-Madison, [2]Biology, Suffolk University

Island populations often exhibit departures from their mainland counterparts in key behavioral, morphological, and life-history traits– a phenomenon termed the "island syndrome". Populations of white-footed mice (*Peromyscus leucopus*) inhabiting two islands within the Boston Harbor archipelago provide compelling examples of this rule, measuring between 40% and 50% larger in body weight than mainland mice. In contrast to the ecological and geographical conditions often thought to drive island syndrome phenotypes, the Boston Harbor islands are situated close to the mainland and harbor both predators and interspecific competitors of *P. leucopus*. In addition, these islands have undergone dramatic environmental transformations over the past 400 years owing to a history of human usage that spans agriculture, industry, and recreation. To characterize the demographic and selective forces that underlie island evolution in this unique system, we performed whole-genome sequencing of wild-caught individuals sampled from two islands and one mainland location. Using site frequency spectra of putatively neutrally evolving single nucleotide polymorphisms (SNPs), we estimated key parameters describing the colonization history of the islands. Our inferred demographic history suggests that divergence times between the islands and mainland largely coincide with the post-glacial formation of the islands. Surprisingly, this allele frequency-based analysis yields little evidence for migration between island and mainland locales. These findings raise the possibility that the Boston Harbor mice evolved concurrently with their changing island environments, providing a unique contrast to examples of rapid evolution of introduced species on islands. We use this inferred demographic history as a null model to evaluate the role that natural selection has played in shaping island-mainland differences. We detected instances of island-specific positive selection by implementing a machine-learning framework based on summaries of nucleotide diversity, allele frequencies, population differentiation, and haplotype structure. We leveraged comparisons of candidate loci between distinct island populations to evaluate the repeatability of adaptive evolution in this unique island system.

781T **Natural selection and random genetic drift in the evolution of genomic regulatory traits: application to epigenetic marks** Leandros Boukas[1], Afrooz Razi[2], Hans T. Bjornsson[3], Kasper D. Hansen[2] [1]Johns Hopkins University/Children's National Hospital, [2]Johns Hopkins University, [3]Johns Hopkins University/University of Iceland

Several molecular traits, such as epigenetic marks and the binding of transcription factors, act as global regulators throughout the genome. It is usually taken for granted that the pattern of the distribution of such traits across the genome reflects pervasive optimization via selection, and this thinking has long permeated efforts to understand their contribution to cellular function. However, we lack a formal framework for testing what genomic patterns are likely to arise under different evolutionary regimes. Without such a framework, it is not possible to know which genomic regulatory traits are indeed under selection, or the extent to which such selection - when present - is pervasive. Methods developed for traditional quantitative traits do not take into account the genome-wide nature of regulatory traits and are thus not ideally suited to answer the above questions.

Here, we develop a neutral null model and a test for directional selection on global regulatory traits. Our approach requires knowing the distribution of the trait of interest across gene-regulatory loci; for example, which promoters are methylated and which are hypomethylated in a given cell type. This allows us to consider variation between genes, and respects that such regulatory traits are often controlled in trans by upstream factors, such as chromatin modifying enzymes.

We then apply our test to epigenetic marks. We consider 3 different marks (DNA methylation, H3K4me3, H3K36me3) and a broad range of genomic compartments (promoters, gene bodies, transcriptional end regions). While we do detect signatures of directional selection, we find that this selection is not pervasive, and there are several genes whose epigenetic state can be explained purely as a result of drift. As an example, we find no evidence for selection promoting DNA methylation at promoters as a global silencing mechanism. We corroborate this with orthogonal empirical data, which reveal that genes with methylated promoters are in fact the genes with the most tolerance to duplications and to expression-level-increasing eQTLs.

A natural question is whether selection on epigenetic marks is purely a passive consequence of selection on gene expression. Our framework allows adjusting for confounders or other traits mediating effects on fitness, thus enabling us to approach this question. We find that, when present, selection on the epigenetic marks under consideration cannot be explained as a passive consequence of selection on expression levels. This supports a causal involvement of these marks in the regulation of gene expression and other processes.

In summary, we present a framework for selection inference on global genomic regulatory traits that is simple, easily applicable and general. We speculate that its application to diverse regulatory traits will help fill an important gap in our understanding of the evolution of this class of molecular traits.

782T **Genetics of color variation in a pair of sympatric butterflies.** Joe Hanly[1,2] [1]Biology, Duke University, [2]Biology, The

George Washington University

Color polymorphisms can serve as a tractable model for the genetic and developmental architecture of traits. Here we investigated continuous color variation in *Colias eurytheme* and *Colias philodice*, two species of sulphur butterflies that hybridize in sympatry. Using quantitative trait locus (QTL) analysis and high-throughput color quantification, we found two interacting large-effect loci affecting orange-to-yellow chromaticity. Knockouts of red Malpighian tubules (red), likely involved in endosomal maturation, result in depigmented wing scales. Additionally, the transcription factor bric-a-brac can act as a modulator of orange
pigmentation. We also describe the QTL architecture of other continuously varying traits, together supporting a large-X effect model where the genetic control of species-defining traits is enriched on sex chromosomes. This study sheds light on the range of possible genetic architectures that can underpin a continuously varying trait and illustrates the power of using automated measurement to score phenotypes that are not always conspicuous to the human eye

783T **Detecting genomic adaptations to freezing environment in right-eye flounder** Prabodh K Bajpai, Xuan Zhuang Biological Sciences, University of Arkansas

Many ectotherms living in subzero environments have evolved remarkable survival strategies to cope with freezing conditions. A notable example is the independent evolution of diverse antifreeze proteins (AFPs) in various cold-water marine fish lineages, such as a subset of right-eye flounder. While we have elucidated the evolution of AFPs in multiple cold-water fish lineages, a comprehensive understanding of freeze adaptation at the genome level across different species is lacking. Right-eye flounders from high latitudinal regions characteristically employ two distinct strategies to avoid freezing in cold waters, with deep-water species relying on undercooling/supercooling, while shallow-water species synthesize Type I antifreeze protein (AFPI). Interestingly, the phylogeny of this family indicates that deep-water species are ancestral to the AFPI-bearing clade. To explore how members within this family, inhabiting in the equivalent subzero waters, employ these two strategies to avoid freezing, we aim to identify genomic signatures of freezing-resistant adaptation using comparative genomics. Our focus extends beyond detecting selection in coding regions to include non-coding regions, which constitute a significant portion of eukaryotic genomes and are implicated in gene expression regulation. Leveraging previously assembled whole-genome sequences in our lab, along with other high-quality genomes available in GenBank, we will conduct selection tests to detect the positive/purifying selection as well as relaxed/intensifying selection on protein-coding sequences. Additionally, we will analyze non-coding regions to discern differences in their evolutionary rates, providing insights into regulatory changes associated with adaptations to freezing environment. By identifying genomic signatures of selection, this study will elucidate the functional classes of genes that have been driving evolution of two distinct freezing avoidance strategies in right-eye flounders. Furthermore, we seek to pinpoint altered biological pathways and gain crucial insights into regulatory regions that undergo modifications during freezing-resistant adaptation.

784T **Evolutionary genomics of trans-specific polymorphisms between cryptic species** Connor S Murray[1], Joaquin Nunez[2], David Bass[3], Madison Karram[1], Madison Doceti[1], Aakrosh S Ratan[4], Alan O Bergland[1] [1]Biology, University of Virginia, [2]Biology, University of Vermont, [3]Cell, Molecular, Developmental Biology, and Biophysics, Johns Hopkins, [4]Center for Public Health Genomics, University of Virginia

The exploration of genetic variation within and between related species represents a fundamental pursuit in genetics. This study aims to discern the evolutionary mechanisms that maintain diversity post-speciation while focusing on trans-specific polymorphisms, loci identical by state across species. The *Daphnia pulex* species complex serves as our focal system, with an emphasis on scrutinizing the abundance of trans-specific polymorphisms between taxa. The central question guiding our investigation is whether trans-specific polymorphisms emerge due to balancing selection or if alternative hypotheses, such as hybridization, incomplete lineage sorting, or convergent de-novo evolution underlie their existence across taxa. To address these hypotheses, we conducted analyses involving over 2,000 genomes of North American and European *D. pulex*, supplemented by several outgroup species. The primary objective of our study was to unravel the prevalence and distribution of trans-specific polymorphisms within the focal species pair, North American and European *D. pulex*. Despite a divergence time exceeding ten million years ago, both North American and European *D. pulex* retain thousands of shared trans-specific mutations. Importantly, the observed number of trans-specific polymorphism cannot be solely explained by hybridization or incomplete lineage sorting. Our analyses demonstrate that most trans-specific polymorphisms arose because of convergent de-novo mutation. However, balancing selection is acting upon recently evolved and ancient trans-specific mutations. In conclusion, our inquiry provides insights into the genetic underpinnings of adaptation and the mechanisms governing the persistence of diversity within the *D. pulex* species complex.

785T **Mathematical properties of allele-sharing dissimilarities** Xiran Liu[1], Zarif Ahsan[2], Tarun K Martheswaran[2], Noah A Rosenberg[2] [1]Brown University, [2]Stanford University

Allele-sharing statistics for a genetic locus measure the dissimilarity between two populations as a mean of the dissimilarity between random pairs of individuals, one from each population. Owing to within-population variation in genotype, allele-sharing dissimilarities can have the property that they have a nonzero value when computed between a population and itself. We consider the mathematical properties of allele-sharing dissimilarities in a pair of populations, treating the allele frequencies in the two populations parametrically. Examining two formulations of allele-sharing dissimilarity, we obtain the distributions of within-population and between-population dissimilarities for pairs of individuals. We use the mathematical results to explain two phenomena observed in human population-genetic data, namely (1) that members of a population can be empirically more genetically dissimilar from each other on average than they are from members of another population, and (2) that when averaging across loci, allele-sharing dissimilarities between pairs of individuals often tend to vary only within a relatively narrow range.

786T **The mechanisms of correlated evolution: Aposematism in Phyllobates poison-dart frogs** Roberto Márquez Ecology and Evolutionary Biology, University of Michigan

A wide array of organisms use conspicuous signals to warn predators of secondary defenses in order to avoid predation, a strategy known as aposematism. In poison-dart frogs aposematism has evolved quickly and dynamically, with multiple independent origins of conspicuously colored and chemically defended lineages. Although the ecological and behavioral processes guiding the evolution of aposematism in poison frogs are relatively well known, the underlying cellular, genetic, and molecular mechanisms remain largely unknown. In this talk I explore the possible molecular, evolutionary genetic, and developmental mechanisms behind the correlated evolution of aposematic coloration and toxicity in Phyllobates poison-dart frogs. Drawing from population genetic, biogeographic, cellular, and developmental perspectives, I focus on how these processes can lead to the convergent evolution of integrated multi-trait phenotypes. Until now, results point to the independent evolution of remarkably similar coloration phenotypes being underlied by changes in different genes, as well as a possible ontogenetic correlation between coloration and toxicity.

787T **Computational advances in inference from allele frequency spectra** Linh Tran[1], Xin Huang[2], Connie Sun[1], Mathews Sajan[1], Sean Davey[1], Travis Struck[1], Ryan Gutenkunst[3] [1]University of Arizona, [2]University of Vienna, [3]Molecular and Cellular Biology, University of Arizona

Inferences of demographic history and natural selection from allele frequency spectra have become a key part of population genomics. But running such inferences can be both computationally expensive and complex. We address these difficulties via two new software packages. First, donni (Demographic Optimization via Neural Network Inference) uses pretrained neural network models to replace the expensive parameter optimization step of demographic history inference, enabling almost instant inference for common models. Second, dadi-cli provides a convenient command-line interface to the software dadi, greatly simplifying inference of demographic history and distributions of fitness effects. In addition, dadi-cli enables distributed computing, including cloud environments. Together, these complementary advances dramatically reduce the computational cost and complexity of inference from allele frequency spectra.

788T **The effect of proto-sex Chromosomes on the reproductive behavior of male house flies (*Musca domestica*)** Farnam Ghaemmaghami[1], Richard Meisel[2] [1]Biology, University of Houston, [2]University of Houston

Female choice should lead to the favored male genotype spreading in the population, reducing genetic variation for male traits. Nevertheless, heritable variation exists in preferred male traits in what has been termed the paradox of the lek. One of the proposed solutions to this paradox is genotype by environment interactions (GEIs). Meaning that a single genotype is not superior over the others over different environmental conditions. Another proposed solution is female preference for unrelated males. When females mate based on their relatedness to males, different females mate with different males and therefore variation is kept. Trade-offs between survival and reproductive success can also contribute to the maintenance of this variation. House flies have a polygenic sex determination system in which the male determining gene (M) can be located on either the Y chromosome (Y^M males) or the third autosome (III^M males). III^M males outcompete Y^M males in population cage competition and mating competition. Yet both genotypes exist in natural populations, showing a clinal distribution, with Y^M males mostly found in the northern latitudes and III^M males more abundant in the southern latitudes. The thermal tolerance and preference of the two types of males is concordant with their geographical distribution. This suggests that temperature might be an influential factor for male performance and consequently could contribute to the maintenance of the two genotypes via GEIs. I am using the housefly system to determine the relative importance of factors maintaining genetic variation of male reproductive behaviors. To this end, I have done population cage experiments with 50% III^M and 50% Y^M males and females from either strain at two different developmental and environmental temperatures (22 and 29℃) and genotype the F1 progeny to see how the ratio of the Y chromosome genotypes changes after one generation. I have also run mating latency assays at the two different temperatures to see whether III^M males mate faster than Y^M males. Finally,

I will design mating competitions to assess female choice and the mating advantage of III^M males over Y^M males. In all my experiments, the background of the female is considered to assess to effect of relatedness to males. This study will help us understand how the interaction between Y chromosome genotype, temperature and female background affects reproductive behavior and how that in turn helps maintain the polygenic sex determination system in house flies.

789T **Genetic diversity loss continues long after habitat destruction ends** Kristy S Mualim[1,2], Jeffrey P Spence[3], Clemens Weiss[3], Meixi Lin[2], Moises Exposito-Alonso[1,2,4] [1]Department of Biology, Stanford University, [2]Department of Plant Biology, Carnegie Institution for Science, [3]Department of Genetics, Stanford University, [4]Department of Global Ecology, Carnegie Institution for Science

A major goal of conservation is maintaining high levels of genetic diversity across species. Recently, there have been calls to begin monitoring genetic diversity levels across species via large scale genotyping. The hope is that such monitoring will detect when too much of a species' genetic diversity has been lost (e.g., due to habitat loss or climate change) and actions can be taken to prevent further loss of genetic diversity. There are several definitions of genetic diversity, but one particularly important measure in terms of various population genetic processes such as adaptation is π, the pairwise heterozygosity. Here we develop mathematical machinery to compute the expected π under complex spatial scenarios. Our modelling, corroborated by realistic simulations and empirical analysis of geospatial genetic data, shows that initially, habitat destruction has almost no impact on π, but in the long term can result in extreme losses in genetic diversity. This result shows that simple monitoring is not enough – genetic diversity will continue to decrease long after habitat destruction ends. Yet, we also show that the time-scale of this genetic diversity loss is quite long – taking a number of generations on the order of the effective population size – giving hope that reversing habitat loss quickly enough can prevent catastrophic consequences.

790T **Biases in ARG-based inference of historical population size in populations experiencing background selection and recurrent sweeps** Jacob I Marsh[1], Parul Johri[2] [1]Biology, University of North Carolina, [2]Biology, Genetics, University of North Carolina

Accurately inferring the demographic histories of contemporary populations using population-genetic approaches provides fundamental insights into species dynamics at both micro- and macro-evolutionary scales. However, alternate evolutionary forces, notably background selection and selective sweeps, can produce genomic signatures that mimic or mask signals associated with historical population size change. While the theoretical biases introduced by linked effects of selection have been well established, it is unclear to what extent typical empirical analyses are susceptible to mis-inference due to these effects. We developed realistic forward simulations of human and *Drosophila melanogaster* populations, including variability of gene density, mutation rates, recombination rates, selection parameters, and historical demographic scenarios, to broadly assess the impacts of selection on demographic inference using the genealogy-based approach *Relate*. Our results suggest analysis of human population histories may be minimally impacted by linked effects of selection. However, in organisms with similar genome architecture, population parameters, and more widespread positive selection, a parameter space relevant to some other mammalian populations, dramatic mis-inference may occur if linked effects of selection are unaccounted for. Finally, we explore the accuracy of demographic inference in populations with compact genomes experiencing widespread selection modelled by our *D. melanogaster* simulations. Our findings identify scenarios in which demographic inference on empirical populations may be compromised by the effects of linked selection and suggest caution when inferring population history in compact genomes.

791T **A diffusion theory approach to model the allele frequency distribution during selective sweeps** Sachin Kaushik[1], Kavita Jain[2], Parul Johri[3] [1]Integrative Program for Biological & Genome Sciences, University of North Carolina at Chapel Hill, [2]Jawaharlal Nehru Centre for Advanced Scientific Research, [3]Department of Biology, Department of Genetics, Integrative Program for Biological & Genome Sciences, University of North Carolina at Chapel Hill

Selective sweeps, resulting from the spread of beneficial mutations in a population, shape the patterns of variation at linked neutral sites. Fixation of a beneficial mutation is expected to reduce nucleotide diversity and skew the site frequency spectrum (SFS) at linked neutral sites, which has been thoroughly investigated using a combined approach of coalescent and diffusion theory in the strong selection regime. We present a simple mathematical framework based on diffusion theory for obtaining the conditional site frequency spectrum at linked neutral sites, both during and immediately post-fixation of moderately or strongly beneficial mutations, assuming no recombination. We extend our approach to include varying dominance coefficients and rates of inbreeding and test the accuracy of our theoretical results when there is interference between segregating beneficial mutations. Our results will be helpful in understanding the effects of sweeps in asexual organisms, sex chromosomes, and in tumor populations

792T **Female mate choice, sexual selection and linkage disequilibrium** Michael Wade Indiana Univ

Linkage disequilibrium across a genome allows the mapping of quantitative traits to genotypes and affects estimates of fundamental genetic parameters like heritability and additive genetic variance. Several processes can create linkage disequilibrium, including epistatic selection, admixture of populations, and assortative mating, while other genomic features affect its rate of decay, including inversions, recombination, gene conversion and inbreeding. Nonrandom mating owing to female mate choice is a type of selective mating that also causes linkage disequilibrium, although it has been less well studied than inbreeding and assortative mating. Using two-locus haploid and diploid population genetic models, I derive the *LD* created by female mate choice between genes for male preferred traits and female preferences for them. Like some types of assortative mating, female mate choice creates selection that changes allele frequencies in males in addition to creating *LD*. In all cases, the stronger a female mating preference, the stronger is the selection on males and therefore, the shorter is the duration of the resulting *LD*, which must be zero when a preferred male allele fixes. This trade-off between the magnitude and duration of *LD* caused by female choice differs from that caused by assortative mating, which can lead to stable non-zero disequilibrium values of *LD*.

793T **Population genetics consequences of fragmentation processes on migration networks** Ryan Chaffee[1], Gili Greenbaum[2], Jaehee Kim[3] [1]Genetics, Genomics, and Development, Cornell University, [2]Ecology, Evolution and Behavior, Hebrew University, [3]Computational Biology, Cornell University

The loss of connectivity between habitable patches, a process known as fragmentation, is a leading cause of declines in animal and plant populations, species extinction, and the loss of biodiversity. Understanding the genetic effects of fragmentation, the loss of connectivity between habitable patches, is crucial in wildlife conservation. Advancements in genomic data enable precise monitoring and management of endangered populations. As human-induced fragmentation through activities such as road construction, deforestation, agriculture, and climate change is increasing globally, comprehending how fragmentation affects genetic variation and population viability becomes vital for conserving and managing wildlife.

To study the genetic consequences of population fragmentation, we developed a computational framework for modeling the fragmentation of migration networks and characterizing the relationships between the properties of the migration networks, the fragmentation process, and genetic measures. Using a structured coalescence model, we analyzed how F_{ST} values and expected heterozygosities change along fragmentation through analytical formulations and numerical simulations. We further assessed critical phase transition points in the F_{ST} and heterozygosity distributions along the fragmentation process to identify optimal intervention strategies that can aid in mitigating the negative genetic effects of population fragmentation.

The analytical formulation of the coalescent theory requires an idealized assumption of migration–drift equilibrium, which is often violated in natural populations. To study the impact of migration–drift disequilibrium on the relationship between migration and the distributions of F_{ST} and heterozygosity, we designed and conducted an agent-based forward time simulation study using SLiM software, which models arbitrarily complex evolutionary scenarios. Then, we further relaxed ecological assumptions of migration symmetry and population size and characterized the effects of simulated fragmentation on these more general systems. Our results highlight the flexibility of our general framework and show its capacity for studying and modeling habitat fragmentation.

794T **Investigating Recombination Rate Variation Through Population-Level Genetic Variation** LyAndra Lujan, Nadia Singh Biology, University of Oregon

Meiotic recombination is pivotal in generating genetic diversity and maintaining genomic stability. This study focuses on unravelling potential genetic determinants that underlie the variation in meiotic recombination rates within crow populations. Through a population genetics approach, we investigate genes associated with meiotic recombination and their potential impact on shaping the genetic landscape of crows.

This study delves into the intricate genetic mechanisms governing meiotic recombination by investigating the genes involved in this fundamental process. Through analysis of the genetic variation within these genes across diverse populations, this research aims to elucidate their impact on the variation of meiotic recombination.

Utilising population-level genomic data from diverse crow species, we conducted a comprehensive analysis of genes that contribute to processes involved in meiotic recombination. By exploring patterns of genetic variation and the evolutionary history of these meiotic recombination genes, we aim to elucidate the factors influencing recombination rate variation in crows.

Carrion crow and hooded crow habitat ranges alternate across Eurasia, creating a longitudinal striped habitat pattern. The overlap of their habitat ranges is small, but others have found hybrid species within these overlapping areas. This study aims to characterise the genetic variation of meiotic recombination genes that could be used to further our understanding of this

interesting pattern of crow habitat ranges.

The findings not only contribute to a deeper understanding of the genetic basis of meiotic recombination rate variation in crows but also provide insights into the divergence of carrion and hooded crows, the broader evolutionary processes within avian populations, and the genetic determinants driving meiotic recombination rate variation in other organisms.

795T **Using long reads to characterize structural variation across diverse species** Danielle Khost[1], Felix Wu[2], Erik Garrison[3], Andrea Guarracino[3], Robin Hopkins[2], Scott Edwards[2], Tim Sackton[2] [1]Informatics, Harvard University, [2]Harvard University, [3]University of Tennessee Health Science Center

Structural variants (SVs) are a broad class of mutations comprising deletions, insertions, duplications and inversions, and are important sources for genetic diversity for many species. In the past their study has been difficult due to technical constraints of short read sequencing. The availability of high-throughput long read sequencing allows improved resolution of these important areas of the genome. Here we discuss two different approaches to characterize SVs using long reads for two highly different sequencing projects: a large-scale pangenome of several scrub jay bird species encompassing 45 individuals, and a population resequencing approach for several *Phlox* wildflower species. The scrub jay genome is relatively small and compact at only ~1 Gb in size, while the *Phlox* genome is 6-7 Gb and is composed of almost 90% repetitive DNA. To construct the scrub jay pangenome, we aligned both haplotypes for all 45 individuals in an all-by-all fashion and constructed a graph using the PanGenome Graph Builder (PGGB), which we then used to call variants. In total we were able to identify >5 million indels across the data set. We compared our results to alternate methods of calling SVs, including using long read mapping and an alternate graph-based approach using Minigraph, and demonstrated general agreement between the different methods. For the *Phlox* species, we mapped Nanopore long reads from 12 individuals across four species to a high quality reference genome, and called SVs using a consensus approach between several different callers. After filtering, we identified ~580k SVs within and between species. We highlight the challenges and considerations for both SV detection methods, as well as contrast the differences in SV dynamics within and between species for these diverse organisms.

796T **Identifying long-lived balanced polymorphisms in humans** Hannah Munby[1], Molly Przeworski[2] [1]Columbia University, [2]Biological Sciences, Columbia University

Modes of natural selection, collectively referred to as “balancing selection,” maintain variation in a population longer than expected under neutrality. In humans, the best understood examples of balancing selection are cases of heterozygote advantage in response to pathogen pressures, which tend to be evolutionary recent. Other clear-cut cases of balancing selection are “trans-species polymorphism,” such as what is seen in the MHC or at the FREM3 locus: variation shared identical by descent between fairly diverged species. While this variation also appears to be maintained by host-pathogen mediated selection, much less is understood about their underpinnings. Here, we search for cases of balancing selection over intermediate time frames, taking an approach that does not rely on a demographic model or assumptions about the specific mode of balancing selection. We use population whole-genome sequencing data from 2504 humans and 59 chimpanzees in order to identify single nucleotide polymorphisms (SNPs) that are identical in the two species. This set of shared SNPs is statistically enriched for older alleles as compared to sets of human SNPs that are matched for allele frequencies, mutation types, and genomic properties, but are not shared with chimpanzees. Thus, the candidate SNPs include variants that have been maintained in humans for longer than expected. This set of candidates includes both previously identified trans-species polymorphism as well as 12 novel loci (at an estimated FDR of 0.3). These loci are either intronic (5) or intergenic (7), and lie near or in a number of genes plausibly involved in host-pathogen interaction. Moreover, an enrichment analysis indicates that our set of candidates is enriched for SNPs in or near glycoprotein genes. Thus, our analysis reveals a number of non-coding loci where variation has been actively maintained by natural selection and thus must be of functional importance, and helps to fill in the gap about our understanding of balancing selection at intermediate time scales.

797T **Trouble in paradox: k-mer diversity scales with population size more than SNP diversity** Miles D Roberts[1], Emily Josephs[2] [1]Genetics and Genome Sciences, Michigan State University, [2]Plant Biology, Michigan State University

At the molecular level, most evolution is expected to be neutral. A key prediction of this expectation is that the level of genetic diversity in a population should scale with population size. However, it was discovered by Richard Lewontin in 1974, and reaffirmed by later studies, that the genetic diversity of natural populations often does not cleanly scale with population size. This observation is known as Lewontin’s paradox. We hypothesize that one contributor to Lewontin’s paradox is that standard genotyping methods underestimate levels of genetic diversity in many species due to their omission of non-SNP forms of variation. To test our idea, we calculated SNP-based and k-mer-based metrics of genetic diversity across 93 plant species, amounting to 133 terabases of DNA sequencing data from over 16,000 plants. We then compared how these different metrics correlated with range size estimates - a proxy of population size - derived from both GBIF occurrence data and expert-drawn range maps. Although we are still refining our analyses, we so far have found that k-mer diversity correlates more

with population size and scales more rapidly with population size than SNP-based diversity estimates. This suggests that the omission of non-SNP forms of variation from diversity surveys may partially explain Lewontin's paradox. The importance of non-SNP variation compared to demography and linked selection in explaining Lewontin's paradox remains an exciting avenue for further research. In the coming months, we will repeat our analyses for approximately 130 plant species.

798T **Asymmetric patterns of postzygotic reproductive isolation within *Mimulus guttatus* species complex** Hagar K. Soliman, Jenn M. Coughlan Ecology & Evolutionary Biology, Yale University

Reproductive barriers are key to species formation and maintenance. Uncovering the evolutionary forces that govern these barriers is crucial for a better understanding of the speciation process. In plants, postzygotic isolation in the form of hybrid seed inviability (HSI) is one of the most common outcomes of interspecific crosses leading to reproductive isolation among various species. Here, we reciprocally crossed different lineages within the *Mimulus guttatus* group to uncover any patterns of HSI. The genus *Mimulus* encompasses a large group of flowering plants including *M. guttatus*, which is particularly considered a model system for speciation studies due to its genetic and phenotypic diversity. Despite their genetic similarity, we found asymmetric patterns of HSI in crosses between the southern and northern lineages collected across the West Coast of North America; crosses between the southern lines of *M. guttatus* as sires and northern lines as dams yielded large, inviable hybrid seeds while the reciprocal direction produced smaller, viable seeds. These results suggest that lineages within the *M. guttatus* group vary in Endosperm Balance Number (EBN). EBN theory could explain why intraploidy-interspecific crosses fail asymmetrically similar to interploidy by assigning each lineage a 'strength' level akin to a plant with a higher or lower physical ploidy number based on how it behaves in a certain cross. We further tested this theory by crossing the 'strong' southern lineages with the 'weak' northern *M. decorus*. Indeed, hybrid seeds were inviable in both directions. Conversely, hybrid seed viability was restored in both directions in crosses between the 'strong' southern lineages and the 'strong' southern *M. decorus*. Overall, our findings may suggest the presence of cryptic lineages within *M. guttatus* and further broaden our understanding of HSI patterns within and between species in the *M. guttatus* species complex.

799T **Investigation of cooperation-cheater games for public-goods driven resources in yeast.** Namratha Raj[1], Supreet Saini[2] [1]Chemical Engineering, IIT Bombay, [2]Indian Institute of Technology Bombay

The phenomenon of cooperation is prevalent at all levels of life. In one such manifestation of cooperation in microbial communities, some cells produce costly extracellular resources that are freely available to others. These resources are referred to as public goods. *Saccharomyces cerevis*iae secretes invertase (public good) in the periplasm to hydrolyse sucrose into glucose and fructose, which are then imported by the cells. After hydrolysis of sucrose, a co-operator retains only 1% of the monosaccharides, while 99% of the monosaccharides diffuse into the environment and can be utilised by any cell. The non-producers of invertase (cheaters) exploit the invertase-producing cells (cooperators) by utilising the monosaccharides and not paying the metabolic cost of producing the invertase. In this work, we investigate the evolutionary dynamics of this cheater-cooperator system. In a co-culture, if cheaters are selected for their higher fitness, the population will collapse. On the other hand, for co-operators to survive in the population, a strategy to increase fitness would likely be required. To understand the adaptation of cooperators in sucrose, we performed a coevolution experiment in sucrose. Our results show that cooperators increase in fitness as the experiment progresses. This phenomenon was not observed in environments which involved a non-public good system. Genome sequencing reveals duplication of several *HXT* transporters in the evolved cooperators. Based on these results, we hypothesize that increased privatization of the monosaccharides is the most likely explanation of spread of cooperators in the population. In another system, we investigate the cheater-cooperator dynamics for melibiose (a glucose-galactose disaccharide). Our results show that in the absence of partial privatization, the results of the public-goods driven game are qualitatively different.

800T **Utilizing a "deep homolog scan" approach to evaluate the theoretical host range of poxviruses** Sophie B Scobell, Michael Chambers, Meru Sadhu National Human Genome Research Institute, NIH

As zoonotic spillovers and epidemics become increasingly common, knowledge of the host range of viruses is vital. Poxviruses have extremely broad and variable host ranges that are largely unknown. One determinant of poxviral host range is the poxvirus protein K3. K3 antagonizes a component of an animal's innate antiviral immune response named protein kinase R (PKR), but a given poxvirus K3 protein only inhibits some animal species' PKRs. We are using a high throughput approach to model the ability of a given poxvirus K3 to inhibit a given animal species' PKR. We are testing several hundred animal species' PKR genes representing a diverse range of animals and mammals. Due to the length of the EIF2AK2 gene that encodes PKR, we design chimeric PKR proteins by replacing a K3-binding region of human PKR with the homologous region from each species in our library. This enables a "deep homolog scan" approach where we can investigate the behavior of critical regions of PKR across diverse phylogeny. In this case, we will characterize both the resilience of PKR and the theoretical host range of 16 poxviruses based on the ability of these virus's K3 proteins to inhibit diverse PKRs in our yeast growth assay. Interestingly,

we find that extremely distantly related species' chimeras yield functional PKR proteins that are also inhibitable by multiple poxvirus K3s. Ultimately, the deep homolog scan approach may be applied to study other host-virus protein/protein interactions in high-throughput, characterizing the potential for infection and giving us new evolutionary insights.

801T **What is the role of epistasis in dictating the evolution of a gene?** Pavithra Venkataraman[1,2], Neetika Ahlawat[1], Sergey Kryazhimskiy[2], Supreet Saini[1] [1]Chemical Engineering, Indian Institute of Technology Bombay, [2]Ecology, Behavior and Evolution, University of California San Deigo

Idiosyncratic epistatic interactions between regions of the genome alter adaptation. However, there exists very little evidence to help predict these effects. I use two model genes in *S. cerevisiae* to understand how interactions between regions of the same gene (intramolecular epistasis) and different regions of the genome (intermolecular epistasis) alter their adaptive trajectories.

To understand how intramolecular epistasis operates, I use a model system in which AmtA (an ammonia transporter in the amoeba *Dictyostelium*) is shifted to the yeast *S. cerevisiae* lacking its native ammonia transporters. The horizontally transferred gene suffers from a localization problem and is non-functional in the new host. Screening of 700 AmtA mutants leads to the identification of 19 variants that restore the functionality of the ammonia transporter. Interestingly, the functionality of the AmtA gene is restored via the acquisition of a small number (one to three) of SNPs. On testing the evolvability of these mutants, we identify that intramolecular epistasis strongly inhibits the evolution of these mutants. I am working towards constructing the intragenic fitness landscape of the AmtA gene in *S. cerevisiae* and understanding the intramolecular constraints on the adaptation of this gene.

Intermolecular epistasis acts in an erratic fashion. I use an ill-adapted allele of Hsp90, a molecular chaperone in *S. cerevisiae*, whose intragenic fitness landscape has been reported to be rugged. Using a high-throughput CRISPR-based method to generate thousands of SNPs in the yeast genome, I am working on understanding how this ill-adapted Hsp90 evolves in different genetic backgrounds, and investigating if there exists any predictability associated with the adaptation of a gene.

802T **Using experimental evolution of hybrid genomes to identify genetic incompatibilities in yeast** Artemiza A Martinez, Gregory I Lang Biological Sciences, Lehigh University

Saccharomyces yeast species are known for their relatively low pre-zygotic barriers to mating, which allows for the occurrence of interspecific hybridization both in the natural environment and within laboratory settings. However, the resultant diploid hybrids are sterile due to significant sequence divergence among *Saccharomyces* yeasts, particularly impeding proper chromosome segregation during meiosis.

Here, we used experimental evolution to investigate the presence of weak but pervasive negative-genetic interactions between nuclear genes within the genomes of sibling species, *S. cerevisiae* and *S. paradoxus.* We generated and sequenced 20 F1 hybrid progeny (using a method developed by *Bozdag, et al. 2019).* Our phenotypic assessments of these interspecific hybrids revealed a spectrum of growth rates at different temperatures and notable fitness defects.

We initiated the evolution of 320 independent populations of haploid and homozygous diploid hybrids for over 2,500 generations in a rich glucose medium. We observed substantial fitness improvements in the evolved populations. We conducted whole-genome sequencing after 1000 generations of haploid hybrids and the 2400 generations of diploid hybrids. We are identifying specific target genes that are mutated more often than we expected by chance when compared to their parental backgrounds, with the goal of identifying advantageous alterations that potentially compensate for genetic incompatibilities within hybrid genomes.

803T **Reversion dynamics of copy number variants: fitness costs versus benefits in the absence of selection pressure** Titir De, Ina Suresh, Pieter Spealman, David Gresham Biology, New York University

Copy number variants (CNVs) -duplications and deletions of genomic regions- are a major source of natural genetic variation between individuals of the same species, leading to rapid adaptive evolution. Under selection pressures, copy numbers of certain genes can increase if they confer a survival advantage, but CNVs can also incur fitness costs associated with increased gene expression. My work investigates the evolutionary dynamics of adaptive CNVs in absence of the selection pressure under which they arose- using the general amino acid permease gene (*GAP1*) in *Saccharomyces cerevisiae* as a model locus. Prior research showed that evolution in glutamine limitation recurrently selects for increase in *GAP1* copy number. However, it is unknown whether acquired CNVs are maintained or lost when this selection pressure is removed.

To study the cost-benefit tradeoffs of CNVs upon removal of a selection pressure, we tested several CNV-containing strains

in nutrient-rich media. These strains emerged from a common one-copy ancestor but differ in CNV structure. In absence of nutrient limitation, we found that CNV strains have lower fitness than their ancestor. We experimentally evolved the strains in rich media for 150 generations, tracking *GAP1* copy number via a fluorescent reporter. Between 60 and 110 generations of evolution, 65% of populations underwent rapid CNV loss. Whole-genome sequencing of multiple clones from each evolved population confirmed that in almost all clones with decreased fluorescence, CNVs underwent reversion to a single-copy genome. Pairwise competition assays show that in rich media, most single-copy revertants have higher fitness than their CNV-containing parent strains. We are performing long-read sequencing to reveal the molecular mechanisms of reversion.

To expand this study to other loci, we are doing similar investigations for CNVs at additional genes, including *MEP2* and *PUT4*. Overall, our results indicate that in the absence of a selection pressure, it is often beneficial for the organism to lose extra gene copies that it had acquired under strong selection, and to revert to a single-copy genome. This suggests that the fitness costs associated with CNVs outweigh their benefits upon removal of the selection pressure under which they arose.

804T **Defining quiescence in Candida albicans** Ozan B Imir, David Gresham Biology, New York University

Candida albicans is the leading cause of fungal infections in the United States and its infection rates are on the rise globally. Studying Candida albicans is vital, as it causes numerous superficial and systemic infections annually, particularly harming immunocompromised individuals. The rising drug resistance in Candida albicans, amidst limited treatment options, urgently demands investigation into the factors behind resistance development. This study zeroes in on quiescence, a critical mechanism that halts growth in response to environmental stimuli.We hypothesized that quiescence could be a contributing mechanism for improved resistance to therapy in Candida albicans. By defining quiescence in Candida albicans, this study aims to provide a deeper understanding of how environmental factors like nutrient availability and downstream signaling pathways could influence fungal tolerance to stress and drug treatments. Our results show that nitrogen and carbon limitations significantly reduce the growth rate of Candida albicans in a concentration dependent manner. Furthermore, we observe an increase in temperature tolerance when Candida albicans is subjected to starvation due to continual culturing. A critical finding of this study is the reduced susceptibility of Candida albicans to fungicidal drugs, specifically caspofungin and micafungin, during quiescent states. Starvation-induced quiescence leads to a dose-dependent increase in cell survival, challenging the efficacy of these commonly used antifungals. Understanding the dynamics of quiescence in Candida albicans opens new avenues for therapeutic strategies and better management of fungal infections, particularly in immunocompromised patients where such infections are most perilous.

805F **Quantification of Environmentally-Dependent Selection via Barcoded Animal Lineage Tracking** Zachary C Stevenson, Ellie A Laufer, Kristin Robinson, Patrick C Phillips Biology, University of Oregon, Institute of Ecology and Evolution

Caenorhabditis elegans is a widely used model organism for studying various biological processes, including neurobiology, aging and experimental evolution. *C. elegans* have many advantages for experimental evolution, such as a rapid life cycle, large brood size, the capacity to freeze and revive populations, self-fertilization reproduction and easy genetic manipulation via CRISPR. We used *C. elegans* as the first genomically-barcoded experimental evolutionary animal model to compete two different strains under various concentrations of ivermectin as a environmental selective pressure. We used N2 as our susceptible strain and JD608 *avr-14*(ad1302), *avr-15*(ad1051), and *glc-1*(pk54), as our resistance strain. We introduced a genomic barcode sequence into lineages of each strain using CRISPR genome editing by TARDIS (Transgenic Arrays Resulting in Diversity of Integrated Sequences), a high-throughput library transgenesis method that allows inducible integration of individual sequences from transgenic arrays into engineered genomic sites. Mixed populations are grown in liquid medium for approximately five generations with various concentrations of ivermectin. By adopting a liquid protocol, we can grow populations in the millions, making this one of the largest animal experimental evolutions to date. We then quantified the relative frequency of each strain in the mixed population by PCR amplification and sequencing of the barcode. Barcode frequencies are then used to measure the fitness of the individual lineages in the population. We found that at low concentration of ivermectin, the sensitive strain holds an advantage, while higher concentrations tend to favor the resistant strain. We also find that the sensitive strain tends to develop slower on increasing ivermectin concentrations, whereas the resistant strain remains mostly stable. We hypothesize this time to reproductive adult is the leading cause of the advantage for either genotype in their respective advantaged conditions. Our results demonstrate that *C. elegans* can be used as a high-throughput barcoded animal experimental evolutionary model to compete different strains and provide replicated high-resolution estimates of fitness within an environmental context.

806F **Multiple distinct evolutionary mechanisms achieve stable coexistence of selfish and cooperative mitochondrial genomes** Bryan L Gitschlag[1], Claudia V Pereira[2], James P Held[3], David M McCandlish[1], Maulik R Patel[3] [1]Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, [2]Takeda Pharmaceuticals, [3]Vanderbilt University

Cells possess multiple copies of mitochondrial DNA (mtDNA). This creates opportunities for selfish mtDNA variants with pathogenic mutations to arise and compete with cooperative (wildtype) mtDNA. These selfish variants are benign at low levels but become costly to host fitness as their frequency rises. Selfish mtDNA variants are thus subject to selection at multiple levels of the biological hierarchy: they compete directly against wildtype mtDNA within host organisms and indirectly due to organismal selection. However, disentangling the relative contributions of the different selection forces operating on mutant mtDNA has proven challenging. We overcome this challenge by combining mathematical modeling with experiments designed to isolate the levels of selection. Applying this approach to many selfish mtDNA genotypes in *Caenorhabditis elegans* revealed an unexpected diversity in the contribution of evolutionary forces. A subset of the mutant mtDNA variants persist at high levels for many generations, despite a heavy host fitness cost, by aggressively outcompeting cooperative (wildtype) genomes within individual hosts. In contrast, some mutant genomes persist by evading organismal selection. Additionally, we also discovered that the mutant variants vary dramatically in their susceptibility to neutral drift. Although these different mechanisms can maintain mutant genomes at similarly high levels, our approach shows how they can give rise to characteristically different distributions of mutant levels among host organisms. Given that mutant levels within an individual represents a key determinant of phenotypic severity, this work outlines an evolutionary theoretic framework for predicting the distribution of phenotypic consequences among individuals carrying a selfish mitochondrial genome.

807F **Limitations of the inference of the distribution of fitness effects of new mutations in partially-selfing populations with linkage** Austin Daigle, Parul Johri UNC Chapel Hill

The accurate estimation of the distribution of fitness effects (DFE) of new mutations is critical for population genetic inference but remains a challenging task. While various methods have been developed for DFE inference using the site frequency spectrum of putatively neutral and selected sites in a population, their applicability in species with diverse life history traits and complex demographic scenarios is not well understood. Selfing is common among eukaryotic species and can lead to decreased effective recombination rates in such populations, increasing Hill-Robertson interference between selected mutations. We employ forward simulations to investigate the limitations of current DFE estimation approaches in the presence of selfing and linked effects of selection. We find that Hill-Robertson interference distorts the site frequency spectrum of highly selfing populations. As a result, inferred deleterious DFEs contain a higher proportion of mildly deleterious mutations compared to the simulated proportions. In addition, the proportion of adaptive substitutions estimated at high rates of selfing is overestimated. Our results better clarify the parameter space where current DFE methods might be problematic and where they remain robust in the presence of selfing and other model violations like departures from semi-dominance, population structure, and uneven sampling.

808F **Intra-genomic coevolution between a DNA satellite and Topoisomerase II triggers a cross-species incompatibility in *Drosophila*** Cara Brand, Mia Levine University of Pennsylvania

Non-coding tandem repeats, called DNA satellites, span megabases of eukaryotic genomes and evolve rapidly. Paradoxically, satellite-rich genomic regions mediate strictly conserved, essential processes like chromosome segregation and genome integrity. A classic resolution to this paradox posits that DNA satellite-associated proteins evolve adaptively to mitigate deleterious proliferation of DNA satellite sequence variants. This intra-genomic coevolution plays out between the 11Mb *Drosophila melanogaster*-specific *359bp* satellite and the DNA repair protein, Maternal Haploid. The *maternal haploid* locus evolves adaptively to ensure that the enzyme, Topoisomerase II (Top2), efficiently resolves DNA entanglements at the *359bp* array. We discovered that, like *maternal haploid*, *Top2* evolves adaptively between *D. melanogaster* and its sister species, *D. simulans.* This signature of adaptive evolution raised the possibility that Top2 also coevolves with *359bp*. To genetically probe this model of coevolution, we overexpressed the diverged *D. simulans* Top2 protein ("Top2[sim]") in the *D. melanogaster* female germline and found that Top2[sim] causes female sterility. These females produced abundant mature eggs, but these eggs failed to hatch, suggesting that maternally provisioned Top2[sim] is toxic to embryogenesis in a *D. melanogaster* background. Furthermore, combining Top2[sim] overexpression with a deletion of the maternal and/or paternal *X*-linked *359bp* array rescues male and/or female embryos in a predictable, *X* chromosome-dependent manner. This finding is consistent with a cross-species incompatibility between the *D. melanogaster*-specific *359bp* array and the maternally provisioned *D. simulans* Top2 protein. Intriguingly, *359bp* entanglement during embryogenesis is reminiscent of a long-studied reproductive barrier between *D. melanogaster* and *D. simulans*. When *D. simulans* females are crossed to *D. melanogaster* males, an unknown, maternally deposited *D. simulans* genetic factor—termed Maternal hybrid rescue (Mhr)—is incompatible with the paternally contributed, *359bp* satellite on the *X* chromosome. This incompatibility manifests as mitotic catastrophe in female embryos. We speculate that Top2 is the elusive *D. simulans* maternal factor, which has remained unmapped for over 100 years. Our study highlights how rapid satellite DNA turnover is as a potent selective pressure shaping genome integrity and may drive the origin of species.

809F **Characterizing allelic changes in Drosophila melanogaster due to selected pressures using various statistical**

models Carmen Martin, Enoch Ng›oma, Elizabeth King University of Missouri

Complex traits are the result of genetic variation within multiple genes and the interaction within their environment. Identifying the genetic basis of these complex traits is highly challenging because the effects at individual loci are difficult to isolate when many genes influence a trait. One approach to identifying the connection between genotype and complex phenotypes is to use experimental evolution to drive phenotypic differences while tracking concurrent changes in allele frequencies in the population. The best approach to identifying and characterizing these changes in allele frequency remains an open question in the research field. Finding different methods to analyze how these allelic changes occur, what are the pressures they are responding to, and how often these changes are continuing into the next generation is a challenge we're exploring. To explore these different methods, we have taken advantage of a highly replicated experimental evolution dataset sampled across multiple time points. This project uses Drosophila melanogaster, a well-characterized model system, to observe how these evolutionary changes are affected by resource availability with three different regimens. We've used twelve replicate lines from an admixed synthetic population (DSPR) and three different resource availability regimens designed to select for evolutionary changes throughout generations. Fluctuating resource availability (FA) to mimic selection during human evolution, constant high resource availability (CH) to mirror the current state of human populations and, a deteriorating resource availability (DA) to mimic the populations with less resources available.

Utilizing different statistical methods to measure the rate of allelic change within each group of our diet regime will give insight into the best methods to identify different types of allele frequency trajectories. In addition, identifying the alleles showing the greatest change over time will identify what variants are more beneficial to different resources available. This type of accurate mapping and analysis gives us insight into how these complex genes are affected while also providing the scientific community with more robust methods to track these highly variable alleles.

810F **Evolved Changes in the Insulin/IGF Signaling Pathway in Response to Diet Regime in Drosophila melanogaster** Elliett J Baca, Joseph Gunn, Elizabeth King University of Missouri

The nutrients within one's environment affect how resources are allocated within organisms as well as their metabolism. The insulin/IGF (insulin-like growth factor) signaling pathway (IIS), a conserved pathway required for metabolism within Drosophila, is crucial for regulating glucose and energy metabolism in the body. Dysregulation of this pathway can result in serious health problems, such as diabetes and obesity. To gain insights into the genetic factors underlying these conditions, we used three experimental evolution treatments to select for divergent resource allocation patterns in Drosophila melanogaster for multiple generations. A fluctuating availability treatment (FA) that goes from standard diet to low yeast to standard, a deteriorating availability treatment (DA) that goes from standard diet to low yeast, and a constant high availability treatment (CHA) that consists of high sugar throughout adulthood. At multiple timepoints during experimental evolution, we assessed how gene expression was evolving in the different diet regimes. First, all lines were placed on a low yeast diet, a standard diet, and a high sugar diet. Then, we collected genome-wide gene expression data of female heads, ovaries, and bodies through RNAseq in generations 5, 10, 19 and 31 to track these expression levels during adaptation to their respective diet regimes. Our primary objective is to find differences in expression within the IIS, a conserved pathway required for metabolism within Drosophila in different generations. This comprehensive dataset provides a picture of how expression in the IIS pathway is affected by selection regime, diet treatment, and organ type.

811F **Functional diversification of duplicate genes in Drosophila** Nathan Duda[1,2], Rob J Kulathinal[1,2] [1]Department of Biology, Temple University, [2]Institute for Genomics and Evolutionary Medicine, Temple University

Gene duplications provide an important source of genetic and functional variation, yet how these duplicates arise, diverge, and are maintained remains an outstanding evolutionary question. In this work, we use a computational evolutionary approach to investigate the functional fate of recently duplicated genes across eight species lineages of Drosophila. Our results reveal a highly dynamic evolutionary history of gene duplication and gene loss across nearly two-thirds of all genes in these eight phylogenetically divergent species. To differentiate between models of functional divergence including neofunctionalization, specialization, subfunctionalization, and conservation, we map the expression profile of ~1,100 lineage-specific duplicates using an extensive and standardized library of transcriptomes derived across seven different tissues (Yang et al. 2018). The genomic structure and function of duplicates was also compared to ~1,600 single-copy ortholog pairs. We find that the majority of duplicates gained a new function in either both copies (specialization) or in only one copy (neofunctionalization). Compared to single-copy genes, duplicates that altered ancestral function experienced strikingly different structural and functional properties, such as lower structural stability and testis-specific expression patterns. This research highlights the role of gene amplification events in generating functional diversity in tissue expression, novel genetic innovations, and species differences at the phenotypic level.

812F **How prevalent are the effects of associative overdominance under realistic evolutionary scenarios?** Parul Johri[1], Brian Charlesworth[2] [1]Biology, University of North Carolina at Chapel Hill, [2]Institute of Ecology and Evolution, School of Biological Sciences, University of Edinburgh

Associative overdominance (AOD) at a neutral locus due to linkage disequilibrium with a closely linked locus subject to mutation to deleterious alleles is expected to occur when $Ns < 1$, $h < 0.5$. It is thus most likely to occur in very low recombining regions, partially selfing populations, or populations that have experienced a recent reduction in population size bottleneck, such as laboratory populations. It has recently been suggested that AOD effects can be quite strong when multiple linked selected sites are involved, and can occur even when $Ns \gg 1$. Using simulations of *Drosophila*-like populations, we examine the effects of AOD on levels of diversity, the distribution of allele frequencies, and patterns of linkage disequilibrium, under a variety of realistic scenarios with multiple selected sites, gene conversion, a distribution of fitness effects (DFE), and strong population bottlenecks, asking when AOD might be detectable in population genomic data. We observe only a weak effect when the DFE is skewed towards highly deleterious mutations but find a substantial effect when the DFE is skewed towards mildly deleterious mutations. However, in the presence of gene conversion and after drastic bottlenecks (mimicking evolution in small laboratory populations), we fail to identify easily detectable effects of AOD. We discuss how commonly made assumptions about modeling fitness at multiple sites can lead to differences in predicted AOD effects.

813F ***Wolbachia* abundance and localization in *Drosophila* hosts diverged up to 50 million years** John P Statz, Brandon Cooper The University of Montana

Many organisms, but especially insects, associate with intracellular microbes. Following establishment, these endosymbionts are typically transmitted vertically through the female germline. Regulation of endosymbiont abundance within and among host tissues is essential for stable host associations. Hosts can contain obligate endosymbionts in specialized organs to regulate them, but facultative endosymbionts are often widely distributed across host tissues in ways that affect host physiology and fitness. This is exemplified by the most common endosymbionts in nature, *Wolbachia* bacteria, that associate with all major insect orders and usually form facultative associations. Using confocal microscopy of whole-mount tissues, we quantify intra- and interspecific variation in *Wolbachia* abundance and localization within *Drosophila* hosts diverged up to 50 million years. We then ask several questions about the relative contributions of host and *Wolbachia* genomes to the regulation of this variation. First, we ask whether germ and somatic tissue regulation is decoupled by testing for covariance and tradeoffs in *Wolbachia* abundance between host tissues. Second, using reciprocally introgressed *Wolbachia*-host genotypes, we test whether *Wolbachia* generally provide positive regulation, while hosts generally provide negative regulation, of *Wolbachia* abundance in germ and somatic tissues. Finally, we ask whether *Wolbachia* abundance and its regulation differ significantly among *Wolbachia* clades that have spread across divergent hosts at differential timescales. Our results contribute to a deeper general understanding of interactions between hosts and the most common endosymbionts in nature. Because *Wolbachia* abundance in specific host tissues covaries with the strength of *Wolbachia*-induced traits like cytoplasmic incompatibility and virus blocking, our results also contribute to a deeper understanding of *Wolbachia* spread in natural systems and to improving the application of *Wolbachia* from *Drosophila* for the biocontrol of diseases and pests in transinfected systems.

814F **'UnLocking' Protein Degradation in *C. elegans*** Adam P Berg[1], Michael Bertram[1], Jakob Faber[1], Henry Giesel[1], Austin Johnson[1], Joseph Kaefer[1], Mitchell Keeling[1], William Keeling[1], Mason Naaman[1], Mawuli Nevis[1], Jordan Scott[1], Connor Wakefield[1], Walter R.P. Novak[1], Erika B Sorensen[2] [1]Wabash College, [2]Biology, Wabash College

Our lab is developing an *in vivo* method to inactivate gene function to better understand normal and disease development. While gene inactivation using RNAi or genetic mutation allows researchers to reduce or eliminate a gene product, these techniques have limitations, including 1) slow protein turnover, which can impair the use of RNAi, and 2) the inability to characterize essential gene function later in development. As a result, effective methods for conditional protein degradation offer a powerful alternative to gene inactivation at the RNA or DNA level. Recent work to develop methods to conditionally deplete proteins (e.g. AID, ZIF-1, PSD) have been effective, but these methods are limiting because they use a single input signal to control protein levels, preventing simultaneous differential control of multiple proteins with only one approach. Our lab is developing the *de novo* 'Latching Orthogonal Cage/Key pRoteins' (LOCKR) technique to control protein degradation in *C. elegans*. LOCKR uses designed protein Switches, which can be genetically fused to proteins of interest, and peptide Keys. The Switch cages a protein sequence, in this case a degron, in the "locked" state (degronSwitch). Upon addition of the Key, the degronSwitch is "unlocked," exposing the degron and causing degradation of degronSwitch fusion protein. Because Keys can be expressed using tissue-specific promoters, degronSwitch fusion protein degradation can be spatially controlled within the animal. In addition, LOCKR Keys and degronSwitches can be designed as orthogonal pairs, allowing for the differential control of multiple proteins simultaneously. To pilot the efficacy of the LOCKR system, we created an inert wrm-Scarlet::degronSwitch reporter via MosSCI. This inert reporter ubiquitously expresses the degronSwitch *in vivo,* and is used to assess tissue-specific

depletion of the degronSwitch in *C.elegans* in the presence of tissue-specific Keys. Similarly, the degronSwitch can be fused to proteins of interest, such as *dhc-1* (dynein heavy chain), a cytosolic motor protein used for molecular transport within a cell, and *snt-1 (*synaptotagimin), an important transmembrane protein for synaptic vesicle exocytosis, to assess the efficacy of degronLOCKR to degrade cytosolic and membrane-associated proteins. This work establishes a novel and tissue-specific method for regulating protein function in *C. elega*ns.

815F **The Evolution Trends of Structural Variants at A Large Population Scale** Jen-Yu Wang, James J. Emerson Department of Ecology and Evolutionary Biology, University of California Irvine

Long-read sequencing simplifies genome assembly and enhances the discovery of structural variants (SVs). Employing *Drosophila melanogaster* as our model, we investigated SV evolution at a population level using over sixty whole-genome long-read sequencing datasets. Comparative analysis of contemporary SV-calling methods yielded population statistics, unfolded site frequency spectra and selection signals.

Traditionally, single nucleotide polymorphisms (SNPs) have been pivotal in inferring phylogeny, population evolution, and disease causation, owing to their detectability with cost-effective methods. Unlike SNPs, SVs, which often span and impact multiple genes, are more likely to influence an organism's phenotype. Despite their significance, SVs have eluded scrutiny due to their complex nature and the limitations of short reads from next-generation sequencing (NGS).

This study bridges the gap by leveraging state-of-the-art long-read sequencing to unveil SVs in *Drosophila melanogaster* populations worldwide. Utilizing Pacific Biosciences and Oxford Nanopore Technology sequences from NCBI SRA, we assessed two major SV detection approaches. Mapping-based programs, though computationally efficient, exhibited lower sensitivity, while assembly-based methods, dependent on well-constructed genomes, captured larger-scale changes. Our validated pipelines are open source on GitHub. Diversity and selection signals varied among SV types, and we sought SVs contributing to population differentiation and chromosomal evolution. Additionally, we constructed the largest *Drosophila* pangenome so far for comprehensive representation of polymorphisms.

816F **Machine learning identifies species differences in *Drosophila* female aggressive behavior that is correlated with reproductive traits** Jennifer M Gleason[1], Eleanor Bath[2] [1]Ecology and Evolutionary Biology, University of Kansas, [2]University of Oxford

Aggression provides access to resources and is therefore a fundamental component of fitness. Species vary greatly in their expression of aggression because of differences in resource needs as dictated by their biology. In *Drosophila melanogaster*, male aggression has been extensively studied, but females are also aggressive and behave in ways that maximize offspring quantity and quality. Notably, mated females are more aggressive than virgin females in the presence of yeast, presumably to increase protein acquisition for eggs. Little is known about the aggressive behavior of females of other *Drosophila* species. We applied machine learning to identify and quantify two aggression behaviors, headbutting and fencing, that are shared among eight *Drosophila* species. The machine learning methods that were validated with *D. melanogaster* work well with these species; however, researcher observations are needed because species-specific agonistic interactions may exist that are missed by relying entirely on classifiers trained on a single species. Across all the species, a notable difference from *D. melanogaster* is that mating sometimes decreased, rather than increased, aggression. We tested if reproductive traits of females (notably ovariole number) are associated with aggression finding that females with high fecundity (more ovarioles) are more aggressive than females with fewer ovarioles in the presence of yeast. Sperm traits (size and number) were weakly correlated with aggression. Species adaptations for reproductive fitness may be associated with aggression in females.

817F **Selection for improved flight performance in laboratory populations of *Drosophila melanogaster*: phenotypic and genomic consequences** Srikant Venkitachalam[1], Natalie Heath[2], Mik Kuehnel[2], Adam Albright[2], Elizabeth King[1] [1]Biological Sciences, University of Missouri, [2]University of Missouri

Quantitative traits are known to result from complex inter- (and intra-) associations of the underlying genetic background and the external environment of organisms. Although mapping genotype-to-phenotype relationships in such complex traits is difficult, long-term experimental evolution studies may help in unravelling some of these connections. This may be achieved through tracking the evolution of the target complex trait, correlated phenotypic markers and sequencing the genomes in the experimentally selected populations, compared to the unselected control populations. In the current study, we have carried out a long-term selection experiment in laboratory populations of fruit flies *Drosophila melanogaster* for a quantitative trait – flight performance. Flight in insects is known to be a complex trait involving nuanced neuromuscular coordination, high motivation to fly, and high energetic demands. Thus, selection for flight performance and subsequent phenotypic and genomic studies would be likely to reveal patterns of genomic evolution and its associative trait changes, pleiotropic relations and shared mechanisms for this complex trait. The selection process involved a custom-built wind tunnel set-up inspired

from a previous study. This wind tunnel was a dark chamber wherein a point of light was provided at one end, and wind resistance increased in gradients closer to the point of light. Each generation, at the start of the selection process, flies were added to the dark end of the chamber. The traversal ability of flies, towards the light and against the wind resistance across this wind tunnel, in a 15-minute window, was defined as their flight performance. In order to determine the true target(s) of selection after 10 generations of adaptation, we performed a factorial experiment wherein we tested the extent of evolution of phototaxis as well as flight performance in the wind tunnel setup. We further measured pre-adult survivorship, dry body mass at eclosion and fecundity as some general phenotypic markers of fitness in the selected populations, compared to their unselected controls. Finally, we sequenced the genome of each population in order to determine how their underlying genetic backgrounds had changed as a consequence of selection.

818F **DEST 2.0: an expanded genomic resource reveals new insights on fly phylogeography and adaptation** Alan Bergland[1], Joaquin Nunez[2], Marta Coronado-Zamora[3], Mathieu Gautier[4], Matin Kapun[5], Sonja Steindl[5,5], Lino Ometto[6], Paris Margot[7], Katja Hoedjes[8], Julia Beets[8], R. Axel Wiberg[9], Giovanni Mazzeo[10], David Bass[10], Dmitri Petrov[11], Paul Schmidt[12], Thomas Flatt[7], Josefa Gonzalez[13] [1]Biology, University of Virginia, [2]University of Vermont, [3]ESCI-UPF, [4]INRAE, [5]National History Museum, Vienna, [6]University of Pavia, [7]University of Fribourg, [8]University Amsterdam, [9]Uppsala University, [10]University of Virginia, [11]Stanford University, [12]University of Pennsylvania, [13]Spanish Scientific Research Council

The rapid development of genomic resources for many species has led to discoveries that contextualize patterns of genetic variation in an ecologically-informed context. To advance our understanding of patterns of genetic variation in Drosophila melanogaster, we present Drosophila Evolution over Space and Time 2.0 (DEST 2.0), a community-generated population-genomic resource that incorporates over 500 population samples (Pool-Seq) of nearly 50,000 flies collected across six continents, some at multiple points in time per year, for over a decade. Using this resource, we characterize the demographic history and signals of adaptive evolution across spatial and temporal dimensions. We identify a suture zone in Europe between the Eastern and Western D. melanogaster clades that aligns with other well known post-glacial secondary contact zones in Europe and present signals of secondary contact between European and African populations in both North and South America as well as Australia. We show that the spatial genetic structure of fly populations is stable over time, but that temporal drift caused by seasonal population contractions is causing populations to diverge over time. We identify signals of adaptation between continents and show that regions of the genome associated with xenobiotic resistance show high levels of spatial differentiation, consistent with heterogeneity in pesticide applications across the world and with independent adaptation to common pesticides. We show evidence of seasonal evolution, using fly samples collected during the spring and fall in localities across Europe highlighting that rapid evolutionary change is a ubiquitous feature of D. melanogaster populations living in temperate habitats. Genomic data, along with standardized metadata is freely available from the DEST website (https://dest.bio). We also provide a genome browser that displays many informative tracks that could be useful for researchers studying the genetic variation and the evolutionary history of this classic model system.

819F **Backyard Evolution – a citizen science project to track seasonal evolution and metapopulation structure in fly communities in backyard compost piles** Megan Stephenson[1], Megan Delamont[1], Abbey Hayes[1], Joaquin Nunez[2,3], Alan Bergland[1] [1]Biology, University of Virgina, [2]Biology, University of Vermont, [3]University of Virginia

Drosophila species living in temperate environments adapt as they evolve to temporal and spatial changes in selection pressure. Contemporary work on adaptive tracking and local adaptation in flies has focused on samples collected from orchards, predominantly from wind-fall fruit. Orchard populations are likely large, permanent and may be subject to temporally varying selective pressures unique to that habitat type, such as pesticide usage. Another habitat for many drosophilids are compost-piles, such as those found in backyards and gardens. This habitat likely harbors smaller and more ephemeral populations that may be subject to different selection pressures than flies from orchards. To test this hypothesis, we initiated Backyard Evolution, a citizen science project to sample flies from backyard compost piles throughout the year. We recruit volunteers primarily from demonstrations at Master Gardeners clubs throughout central Virginia, although volunteers from across the USA have contributed. Backyard Evolution has been running every summer since 2020 and these samples will be combined with seasonal sampling at multiple orchards throughout central Virginia. In total, we have worked with 45 volunteers and collected flies from a half dozen orchards, yielding over 56,000 flies from over 14 species. We show that the community composition of orchard and compost pile samples are different, with more species present in compost piles than in orchards. We will be conducting whole-genome resequencing of samples from selected species to test for: (1) adaptive differentiation between orchards and compost piles; (2) differences in population persistence and turnover between species and habitat types; and (3) signals of parallel seasonal evolution in highly divergent Drosophila species. Backyard Evolution is always taking new recruits so stop by if you would like to learn how to participate.

820F **Intraspecific variation in heat-shock transcriptional response in *Drosophila melanogaster*** Nikale Pettie[1], Ana Llopart[1,2], Josep Comeron[1,2] [1]Department of Biology, University of Iowa, [2]Interdisciplinary Program in Genetics, University of

Iowa

An organism's ability to respond to stressors is essential for survival. One of the trademark features of heat stress response is a significant increase in expression of heat shock proteins (HSPs), such as the evolutionarily conserved chaperone Hsp70, after heat shock. Here, we characterized the transcriptional response to acute heat shock (39°C, 5 minutes) in *D. melanogaster* strains from different geographic locations: Raleigh, NC, which experiences a wide range of temperatures (with average lows and highs varying more than 30°C between winter and summer) and Fiche, Ethiopia, with much more limited seasonal variation. We generated total RNA-seq data from ovaries immediately after heat shock as well as after recovery for 60 and 240 minutes. As expected, transcripts for HSPs significantly increased in strains from both populations. There were, however, critical differences between populations in the response of the nonsense-mediated mRNA decay (NMD) pathway as well as in the total number of genes that change transcript levels after heat-shock. The NMD is a conserved and highly selective RNA degradation pathway that also serves as a major regulator of the unfolded protein response (UPR), modulating stress responses in species across the phylogenetic scale. Our results, therefore, suggest population-specific NMD regulation in association with the different natural environments. To study the long-term effects of recurrent heat stress, we also investigated transcriptional responses after multigenerational heat-shock.

821F **Segregation Distorter requires Overdrive for gamete elimination** Jackson Ridges[1], Jackson Bladen[1], Raphaëlle Dubruille[2], Benjamin Loppin[2], Nitin Phadnis[1] [1]School of Biological Sciences, University of Utah, [2]Laboratoire de Biologie et Modélisation de la Cellule, University of Lyon

Intragenomic conflict involving selfish chromosomes is a powerful force in evolution that shapes the evolution of genomes, cells, and species. Segregation distorters that operate in the male germline work through the selective elimination of sperm bearing the competing homologous chromosome. Although selfish systems are known across species, the molecular mechanisms underlying selfish chromosomes remain mysterious. *Overdrive (Ovd)* is necessary for both segregation distortion and male sterility in *Drosophila pseudoobscura Bogota-USA* hybrids. Here, we show that *Ovd* is a non-essential gene that is also required for the selfish action of the nonorthologous selfish system *Segregation Distorter* in *D. melanogaster*. In particular, *Ovd* is required for the targeted elimination of competing gametes by SD. Our results suggest that tricking germline checkpoints may be a common mechanism of selfish chromosomes.

822F **Egg laying behavior in response to CO_2 exposure in *D. suzukii* subgroup** Sasha A Mills[1], Alice Gadau[1], Xin Yu Zhu Jiang[2], Li Zhao[1] [1]The Rockefeller University, [2]Hunter College

All organisms interpret, understand, and react to their environments via an array of inputs to their nervous system. Studying these inputs and the corresponding behaviors may elucidate the genetic basis of these behaviors. The model system Drosophila provides an excellent opportunity to study both the genetic basis and behavioral consequences of exposure to stimuli. Several Drosophila species such as *D. melanogaster* are known to prefer to oviposit on rotten fruits, but the closely related pest species *D. suzukii* and its sister species *D. subpulchrella* have adapted a preference for ripe fruit. This preferential difference is especially interesting to study because ripe fruits have a different odor, texture, and chemical composition than rotten fruits. Ripe fruit also emit more carbon dioxide (CO_2) because of respiration during ripening. In this study, we investigated how CO_2 influences the egg-laying preference of the aforementioned Drosophila species. We used a four-field olfactometer to conduct two-choice egg-laying trials in the presence and absence of CO_2 with *D. melanogaster*, *D. subpulchrella*, and *D. suzukii*. We looked at both positional and oviposition preference and found that the presence of CO_2 severely inhibits egg-laying in *D. melanogaster* but is not aversive to the other two species. We hypothesized that *D. subpulchrella* and *D. suzukii*'s adaptation to higher CO_2 levels may be due to changes in the CO_2 receptors coding region or cis-regulatory element. To substantiate this hypothesis, we designed transgenic D. melanogaster lines that express the *D. subpulchrella*, *D. suzukii* or *D. melanogaster* Gr63a Coding Sequence (CDS) under each species cis-regulatory element. We found that the *D. subpulchrella* CDS shows an increased preference to CO_2 while flies with the *D. suzukii* CDS show an extreme aversion to CO_2. The *D. suzukii* cis-regulatory elements returned some preference for CO_2 in the transgenic lines. This finding could be critical in explaining why and how *D. suzukii* prefer ripe rather than rotten fruit.

823F **Investigating ZAD Gene Evolution on Muller D** Andrew M Arsham[1], Anthony D Cole[2], Tom Giarla[3], Anthony M Howard[2] [1]Department of Biology, Bemidji State University, [2]Bemidji State University, [3]Siena College

The ZAD gene family is defined by the presence of an N-terminal zinc finger associated domain (zf-AD or ZAD), often accompanied by one or more C-terminal C2H2 zinc finger domains. There are more than 90 ZAD proteins in *Drosophila melanogaster* and many are implicated in the regulation or function of heterochromatin. While a small handful of ZAD genes are present in all Arthropods, the family has expanded dramatically in Insects since their divergence from Crustaceans. Accurate characterization of large gene families has historically been hampered by the absence of high quality gene

annotations the difficulty of distinguishing orthologs from paralogs within expanding gene clusters. Here we use recently released high quality gene models generated by the NCBI Eukaryotic Genome Annotation Pipeline to characterize ZAD family evolution across several insect orders. We retrieved all NCBI-annotated transcripts for 257 refSeq Insect genome assemblies and searched their predicted proteins for the presence of ZADs. Preliminary analysis reveals that the ZAD family has expanded by almost 40 fold during insect evolution, from fewer than 20 in some hemipteran aphids to over 600 in the dipteran mosquito *Aedes albopictus*. Detailed analysis of all ZAD genes on the Muller D Element (corresponding to D. melanogaster chromosome 3L) in 21 species of the *Drosophila* genus reveals clade-specific expansions in D. *virilis* and *novamexicana* (25 Myr), the D. *Takahashii* clade, and most notably in D. *kikkawai* where a single ZAD gene appears to have generated a cluster of 16 paralogous copies within the last 8 million years. Interestingly the first two expansions consist mostly of genes with a single coding exon, suggesting possible retroduplication, while the genes of the D. *kikkawai* expansion are all highly expressed predominantly in the embryo. The duplication of ZAD genes appears to follow a similar pattern to olfactory and gustatory receptors and detoxification enzymes in that some phylogenetic branches are virtually unchanged in 50 million years while others spawn multiple independent clade-specific expansions, forming phylogenetic "blooms" suggesting that some members of the gene family are inherently duplication-prone and others are not.

824F **Molecular evolution of a maize hybrid barrier over 12 million years** Elli Cryan[1,2], A Garnet Phinney[1], Arun S Seetharam[3,4], Matthew M S Evans[5], Daniel J Kliebenstein[2], Jeffrey Ross-Ibarra[1] [1]Evolution & Ecology, UC Davis, [2]Plant Sciences, UC Davis, [3]Ecology, Evolution and Organismal Biology, Iowa State University, [4]Genetics, Development and Cell Biology, Iowa State University, [5]Plant Biology, Carnegie Institution for Science

The *Zea mays* species includes three main subspecies: domesticated maize (*mays*), and two teosinte subspecies (*parviglumis* and *mexicana*). All three can readily hybridize, yet remain distinct. Mating incompatibility loci provide one mechanism that can allow populations to maintain reproductive isolation. In *Zea mays*, three complex mating incompatibility loci encode genes that disrupt directional pollen tube growth down the silk. When a maternal plant receives pollen with incompatible alleles, fertilization is impeded, creating a prezygotic reproductive barrier. However, this barrier is not complete. Infrequent fertilization of incompatible gametes facilitates introgression of incompatibility genes into other populations. Previous modeling shows that each factor should only undergo brief periods of strong selection, on a timescale shorter than time to speciation in this clade. Against this expectation of transient benefit, we find evidence of syntenic gametophytic factor loci across modern maize lines, *Zea mays* teosinte subspecies, other members of the *Zea* genus and Tripsacinae subtribe, and species as diverged as *Sorghum bicolor*. All of the loci display presence absence variation and copy number variation. To reconstruct the evolutionary history of these complex loci, we classify haplotype diversity at all three loci in the over 30 *Zea mays* genomes, identify syntenic loci in related species, construct gene trees of known and candidate functional genes, and analyze rates of molecular evolution. We find new evidence of potentially functional reproductive barrier loci and genes in lineages that have been estimated to be twelve million years diverged. We also find evidence of loci driving epigenetic silencing of interacting loci. These loci may have played a role in *Zea* genus speciation.

825F **Inferring Distributions of Fitness Effects of Wild House Mice from Allele Frequency Spectra** Olivia Fernflores, David Castellano, Emanuel Fonseca, Travis Struck, Ryan Gutenkunst University of Arizona

The distribution of fitness effects (DFE) of new mutations is a key input into the evolutionary process. We aim to infer the DFE among multiple populations of wild house mice, so that the extensive knowledge of mouse molecular biology can be leveraged to understand the biological basis of the DFE. To infer the DFE, we first use synonymous mutations to infer a model of demographic history. Here we present preliminary demographic history inferences for populations from Iran and France. We find the best models are those that include a distinct split between the two populations and account for inbreeding between closely related individuals.

826F **On the demographic history of the Western European house mouse, *Mus musculus domesticus*** Kennedy Agwamba Computational Biology, UC Berkeley

Human commensal, *Mus mus domesticus*, is native to the European continent, with a range extending from the Middle East to western Europe by the end of the Iron Age. Wild populations of *M. m. domesticus* are now distributed across Africa, the Americas, and Oceania, a range notably consistent with the global migration patterns of western Europeans that began in the early 16th century. Despite its standing as the premier mammalian model organism for biomedical, ecological, and evolutionary research, important details surrounding the population history of wild house mice remain a mystery. To investigate patterns of genetic structure and infer the demographic history of the Western European house mice, we analyze a collection of 183 mice sampled from western Europe and the Americas, including 59 new whole genome sequences from historically relevant regions of western Europe. Unsupervised clustering analysis groups all samples by geographic location, uniquely identifying a northern European, Mediterranean, and Atlantic Iberian population clade among European samples. Admixture graphs reveal

the Atlantic Iberian clade to be sister to all populations of house mice in the Americas, and a migration edge from the UK to the base of the North America clade indicates a distinct secondary introduction of house mice to the Americas. Demographic models reveal that American populations diverged largely within the last 500 years, consistent with the timing of European colonization history in the Americas. Altogether, these results provide clarity around the recent introduction of Western European house mice to the Americas, highlighting the effects of human migration and global colonization on the concurrent spread of an invasive human commensal.

827F **Differential evolution of gene sequences, expression profiles, and protein structures** Antara Anika Piya[1], Raquel Assis[2] [1]EECS, Florida Atlantic University, [2]Florida Atlantic University

Evolution of genes can occur through modifications at their DNA, RNA, or protein levels. However, the role of each of these changes in genic evolution is currently unclear. Here we address this problem by comparing divergence rates of protein-coding sequences, multi-tissue expression profiles, and protein structural flexibilities in rodents, primates, and grasses. Our analysis reveals that divergence rates are generally lowest for sequences and highest for protein flexibilities in all taxa, consistent with the idea that higher levels of biological organization are closer proxies for the functions on which selection acts. However, divergence rates across these levels are minimally correlated, suggesting that evolutionary targets vary across genes. Indeed, different functions are enriched in genes with high sequence, expression, and protein structural divergence rates. Together, these findings support the hypothesis that evolutionary targets of genes depend on their functions, shedding light on how selection may alter different levels of biological organization in animals and plants.

828F **Effect of chloroplast size and abundance on photosynthesis in diploid vs. polyploid wheat** Damilola Odumade, Joel Sharbrough Biology, New Mexico Institute of Mining and Technology

The plant genome is partitioned into three separate compartments: nucleus, plastid, and mitochondrion. Rubisco (Ribulose-1,5-biphosphate carboxylase/oxygenase) performs carbon fixation in plant chloroplasts and, similar to other jointly encoded plastid-nuclear enzyme complexes, consists of an equimolar ratio of nuclear-encoded small subunits and chloroplast-encoded large subunits. Elevation of nuclear genome copy via whole-genome duplication perturbs the delicate stoichiometry between the nuclear and plastid genomes, potentially affecting the assembly dynamics and/or abundance of plastid-nuclear enzyme complexes like Rubisco. In response to such profound genomic changes, recent work has shown that polyploids exhibit elevated chloroplast abundance and chloroplast genome copy number per cell to compensate for the perturbed stoichiometric imbalance. Consequently, polyploids are expected to exhibit higher photosynthetic capacity than related diploids. We are testing this hypothesis at the genomic and phenotypic level in diploid (*Triticum urartu*, *Aegilops speltoides*) vs. polyploid wheat (tetraploid pasta wheat – *Triticum turgidum*, hexaploid bread wheat – *Triticum aestivum*). At the genomic level, we determined the ratio of the nuclear: chloroplast genome copy number per cell using quantitative PCR. Phenotypically, we imaged chloroplasts in the mesophyll of leaf tissue using light microscopy and counted and measured chloroplast size with ImageJ. We are currently measuring photosynthetic rate using an Oxigraph+ respirometer and will use linear models to test for relationships between the genomic, tissue, and organismal level traits. Together, the data that will be produced during this project will provide important evidence regarding the question of how polyploids are able to overcome the constraints of polyploidy and remain an evolutionary success.

829F **Genomics of Brazilian howler monkeys reveals adaptation to malaria** Katherine McVay[1], Erick Figueroa-Ildefonso[1], Katharine Korunes[1], Luana Portela[2], Patricia Domingues de Freitas[2], Victor Y. Guimarães[3], Ligia S. L. Silveira da Mota[3], Cauê Monticelli[4], João Valsecchi[5], Fernando A. Perini[6], Alcides Pissinatti[7], Amy Goldberg[1] [1]Duke University, [2]Federal University of São Carlos, [3]São Paulo State University, [4]São Paulo Wildlife Coordination, [5]Mamirauá Sustainable Development Institute, [6]Federal University of Minas Gerais, [7]Primatology Center of Rio de Janeiro

Malaria is one of the strongest selective pressures in human evolution, with dozens of genes implicated in adaptation to different Plasmodium parasites. Over 30 Plasmodium species infect non-human primates, including regular parasite sharing with humans. Plasmodium is hypothesized to have arrived in South America ~500 years ago through European Colonization and the Trans-Atlantic Slave Trade. Thus, South American primates provide an opportunity to understand adaptation in a malaria-naïve primate system. In particular, howler monkeys (genus Alouatta) are the primary reservoirs for P. simium, a vivax-like zoonotic malaria infecting humans in southern Brazil, and regularly share P. malariae/brasilianum with humans. We conducted whole-genome sequencing on 88 howler monkeys (average coverage ~11X) to understand broad patterns of shared or unique adaptations to malaria between humans and multiple howler species. Based on 43 host genes known to interact with malaria (adapted from Ebel et al. 2017, PLoS Genetics), we find preliminary evidence for shared and species-specific variation and adaptation in genes associated with red blood cell structure and invasion using a combination of allele-frequency and haplotype-based summary statistics. Additionally, we detect species-level infections with Plasmodium parasites in 14 howler individuals using KrakenUniq, with a higher proportion of infections in the Legal Amazon than other regions. We further

reconstruct broader population structure and demographic history of 4 howler monkey species from across Brazil, finding differences in rates of inbreeding and population size by species. Despite prior ecological suggestions of hybridization, we do not detect evidence of gene flow in our sample.

830F **Multilocus phylogenetic tree estimation using topic modeling** Marzieh (Tara) Khodaei[1], Scott Edwards[2], Peter Beerli[1] [1]Scientific Computing, Florida State University, [2]Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard

Inferring the evolutionary history of species or populations employing multilocus analysis is gaining ground in phylogenetic analysis. We developed an alignment-free method to infer the multilocus species tree, which is implemented in the Python package TopicContml. The method operates in two primary stages. First, it uses probabilistic topic modeling (specifically, Latent Dirichlet Allocation or LDA) to extract topic frequencies from k-mers, which are in turn derived from multilocus DNA sequences. Second, these extracted frequencies serve as an input for the program Contml in the PHYLIP package, which is used to generate a species tree. We evaluated the performance of our method with two datasets: a biological dataset with 14 DNA sequence loci from 78-92 individuals from 9 locations and simulated datasets. Both our empirical results and simulated data suggest that our method is efficient and statistically accurate. We also assessed the uncertainty of the estimated relationships among clades using a bootstrap procedure for both unaligned and aligned multilocus sequence data. TopicContml proves a valuable contribution to the computational toolkit for phylogenetics by efficiently constructing evolutionary trees without sequence alignment. It is capable of efficiently extracting the tree by inferring large multilocus dataset, and to our best knowledge, there are no current software packages for constructing multilocus phylogenetic trees using unaligned input data.

831F **Inference of the structured coalescence in the fractional coalescent framework.** Peter Beerli[1], Somayeh Mashayekhi[2] [1]Florida State Univ, [2]Mathematics, Kennesaw State University

We introduced the fractional coalescent to discuss potential heterogeneity affecting offspring number within a single population in 2019. Here, we extended this framework to infer differences of heterogeneity among different populations that are linked by recurrent migration. Our approach uses multilocus DNA data as input and estimates parameters of a structured coalescent model with multiple populations linked by migration. Each population has an additional parameter alpha, that changes according to the magnitude of heterogeneity within the population.

For simulation testing of our inference program, we created a 2-population extension of the Nest Site Model, introduced by Wakeley, to generate synthetic DNA data. This simulated data is then used to estimate population sizes, immigration rates, and the parameters alpha that describe the potential differences in response to environmental heterogeneity. We report accuracy and timing for various numbers of loci and different parameter settings.

832F **Differentiating mechanism from outcome for ancestry-assortative mating in admixed populations** Dashiell J Massey[1], Zachary A Szpiech[2,3], Amy Goldberg[1] [1]Evolutionary Anthropology, Duke University, [2]Department of Biology, Pennsylvania State University, [3]Institute for Computational and Data Sciences, Pennsylvania State University

Non-random mating is an important source of genetic structure in natural populations. Empirical studies across multiple animals, including humans, have found positive correlations in trait values between mates, potentially confounding genome-wide association studies, selection scans, and demographic inference. In particular, within recently admixed human populations – who derive ancestry contributions from multiple source populations – previous work has observed a correlation in global ancestry proportion between spouses, known as ancestry-assortative mating. Population genetic models of ancestry-assortative mating typically calculate the likelihood of mating using ancestry proportion. Here, we use whole-genome forward simulations in SLiM to interrogate the relationship between the mechanistic process of mate choice and observed outcomes for the potential correlation in ancestry between mating pairs. We consider commonly-used ancestry-based mate choice models alongside a discrete sociological model based on group identity. In humans, sociodemographic factors strongly influence mating, and are often correlated with genetic ancestry, providing the opportunity for social group identity to serve as the link between ancestry and mate choice. We find that even a mate-choice model that does not explicitly include preferences based on ancestry, but rather uses group identity as a proxy, can produce correlations in ancestry between mating pairs, with similar results for summaries of global and local ancestry. That is, multiple potential mate-choice processes can produce similar ancestry correlation plots, suggesting caution in interpretation of empirical data. Conversely, even when the underlying process involves biased mate choice, the observed outcome may not show a correlation in ancestry because without further migration, some common models do not maintain sufficiently high variance in ancestry proportion to sustain ancestry-assortative mating over tens of generations.

833F **Inferring Demographic History in the Presence of Low-Coverage Sequencing** Emanuel Masiero da Fonseca, Ryan Gutenkunst Molecular and Cellular Biology, University of Arizona

The emergence of low-coverage genome sequencing as a cost-effective approach has opened avenues for the comprehensive analysis of large cohorts. Nevertheless, the inherent reduction in heterozygous genotypes associated with this strategy introduces a notable source of bias in downstream analyses, including demographic history inference. In response to this challenge, we developed a novel probabilistic model expressly designed to reduce the distortions induced by low-coverage sequencing and implemented it in the inference software dadi. We assessed the accuracy of this framework by examining simulated low-coverage datasets that portrayed two distinct demographic scenarios: an exponential growth model and a strict isolation model. We found that the incorporation of distortion correction led to a close alignment between the inferred and true demographic parameters. Conversely, failing to consider the distortion introduced by low-coverage sequencing resulted in a notable bias during parameter estimation. To complement our simulations, we conducted real-data analyses, downsampling the 1000 Genomes Project data and analyzing low-coverage data from three distinct fish species. The application of our probabilistic model to these datasets further validated the efficacy of our correction method, demonstrating its utility in addressing challenges associated with reduced heterozygosity in real-world scenarios. In conclusion, our research underscores the critical importance of mitigating bias introduced by low-coverage sequencing through the application of a probabilistic model. By implementing this correction, we not only address the challenges associated with reduced heterozygosity but also enhance the accuracy of demographic parameter inference, thereby increasing the reliability of population genetic studies in the genomic era.

834F **Co-existing strains drive rapid and reversible genetic turnover in antibiotic treated human gut microbiomes** Sophie J Walton[1], Katherine S Xue[2], Daniel PGH Wong[3], David A Relman[3], Dmitri A Petrov[2], Benjamin H Good[4] [1]Biophysics, Stanford University, [2]Biology, Stanford University, [3]Stanford University, [4]Applied Physics, Stanford University

Transient perturbations can rapidly alter the composition of microbial communities like the human gut microbiome, but the evolutionary processes that underlie this collective response are still poorly understood. In particular, it remains unclear to what degree evolutionary change is driven by selection on de novo mutations rather than displacement of dominant strains by distantly related strains of the same species. To address this gap, we examined longitudinal metagenomic sequencing data from the gut microbiomes of 22 participants who took a 5-day course of the antibiotic ciprofloxacin. These participants experienced transient or permanent shifts in microbiome composition at the onset of antibiotic treatment, which suggested antibiotics induced transient selection pressures on microbiota populations. We found few examples of de novo mutations in the core genome that swept during or after antibiotic perturbation, even in populations that expanded substantially during antibiotics and persisted at a high frequency. However, we found many instances of strain displacement by distantly related populations of the same species events during antibiotic administration, which were often driven by expansions of pre-existing strains within a host from low to high frequency, rather than colonization by external strains. One third of the displaced strains reverted back to high frequency after the antibiotic perturbation ended, such that the strain after antibiotics closely resembles the pre-antibiotic composition even if the host microbiome composition shifts to an alternative stable state. To investigate whether this reversible genetic turnover is driven by selection, we are simulating neutral population genetic models to examine whether demography alone can drive the observed frequency trajectories. These results suggest that the coexistence of multiple strains of the same species in a person's gut microbiome can drive reversible genetic turnover in response to transient perturbations without core genome evolution.

835F **Enrichment of sex-biased genes associates with the origins of multiple neo-sex chromosomes in Danaini butterflies** Pablo Mora[1,2], Monika Hospodářská[2], Anna Voleníková[2], Petr Koutecký[2], Jana Štundlová[2], Martina Dalíková[2,3], James Walters[3], Petr Nguyen[2] [1]University of Jaén, [2]Faculty of Science, University of South Bohemia, [3]Ecology & Evolutionary Biology, University of Kansas

Sex chromosomes play an outsized role in adaptation and speciation, and thus deserve particular attention in evolutionary genomics. In particular, fusions between sex chromosomes and autosomes can produce neo-sex chromosomes, which offer important insights into the evolutionary dynamics of sex chromosomes. Here we investigate the evolutionary origin of the previously reported *Danaus* neo-sex chromosome within the tribe Danaini. We assembled and annotated genomes of *Tirumala septentrionis* (subtribe Danaina), *Ideopsis similis* (Amaurina), *Idea leuconoe* (Euploeina), and *Lycorea halia* (Itunina) and identified their Z-linked scaffolds. We found that neo-sex chromosomes shared by *Danaus* spp. arose in a common ancestor of Danaina, Amaurina, and Euploina. We also identified two additional independently evolved neo-Z chromosomes in *I. similis* and *L. halia.* We further tested a possible role of sexually antagonistic selection in sex chromosome turnover by analyzing the genomic distribution of sex-biased genes in *I. leuconoe* and *L. halia*. The synteny blocks involved in the fusions are enriched in sex-biased genes, consistent with the hypothesis that this could facilitate fixation of neo-sex chromosomes. This suggests a role of sexual antagonism in sex chromosome turnover in Lepidoptera. The neo-Z chromosomes of both *I. leuconoe* and *L. halia* appear fully compensated in somatic tissues, but the extent of dosage compensation for the ancestral Z is variable across tissues and species.

836F **The unreasonable effectiveness of Graph Convolutional Networks in population genetic inference** Logan S Whitehouse[1], Dylan D Ray[1], Daniel R Schrider[2] [1]University of North Carolina, [2]Department of Genetics, University of North Carolina

In recent years researchers have begun adapting machine learning methods to population genomics, including the development of deep neural networks that act directly on population genetic alignments. In general, these deep learning methods show enormous promise for more accurate inference. There have also been a number of exciting recent advances related to the development and deployment of novel tree-based representations of genotype data, including the release of several methods that seek to rapidly infer the sequence of trees along a recombining chromosome for large cohorts. Importantly, these tree sequences not only represent a hypothesized evolutionary history of a set of genomes, they also enable highly compressed storage of population-scale sequence data. These advancements have led some researchers to explore the possibility of using tree-sequences, or summaries thereof, as input to machine learning methods. However, one may notice that this approach appears to be an especially severe violation of Vapnik's aphorism: "When solving a problem of interest, do not solve a more general problem as an intermediate step." Here, the intermediate step is to first infer the entire evolutionary history of every locus across the genome for a population sample, prior to, say, asking whether locus *A* has experienced a recent selective sweep. Another potential caveat with such approaches is that tree-sequence inferences are error-prone, and it is unclear to what extent using erroneous trees as input will hinder subsequent inference. With these potential issues in mind, we sought to evaluate the general utility of tree sequences for population genetic inference. To this end, we trained graph convolutional networks (GCNs) to take inferred tree sequences as input and perform four different tasks: detecting recent selective sweeps, detecting introgressed loci, inferring recombination rates, and inferring the parameters of a simple demographic model. We compared the performance of this GCN to a convolutional neutral network (CNN) that we previously showed to achieve excellent performance on these problems. Surprisingly, we found that for each task the performance of the GCN was comparable to that of the CNN. This implies that inferred tree sequences can be used as input for accurate downstream inference for more specific population genetic questions. Importantly, because we used inferred tree sequences that were likely reconstructed with a fair amount of error, our results imply that erroneous tree sequences do not preclude accurate subsequent inference so long as the model has been trained on tree sequences inferred in the same manner. Overall, we conclude that tree sequence-based machine learning methods may prove to be just as powerful and flexible for population genetic inference as CNNs, and have the potential to be more computationally efficient as well.

837F **Inference of the demographic histories and selective effects of human gut commensal microbiota over the course of human history** Jonathan C Mah[1], Kirk E Lohmueller[2], Nandita Garud[3] [1]Bioinformatics Interdepartmental Program, University of California, Los Angeles, [2]Ecology and Evolutionary Biology, University of California, Los Angeles, [3]Bioinformatics, University of California, Los Angeles

Despite the importance of gut commensal microbiota to human health, there is little knowledge about their evolutionary histories, including their population demographic histories and their distributions of fitness effects (DFE) of new mutations. Here, we infer the demographic histories and DFEs of 27 of the most highly prevalent and abundant commensal gut microbial species in North Americans over timescales exceeding human generations using a collection of lineages inferred from a panel of healthy hosts. We find overall reductions in genetic variation among commensal gut microbes sampled from a Western population relative to an African rural population. Additionally, some species in North American microbiomes display contractions in population size and others expansions, potentially occurring at several key historical moments in human history. DFEs across species vary from highly to mildly deleterious, with accessory genes experiencing more drift compared to core genes. Within genera, DFEs tend to be more congruent, reflective of underlying phylogenetic relationships. Taken together, these findings suggest that human commensal gut microbes have distinct evolutionary histories, possibly reflecting the unique roles of individual members of the microbiome.

838F **Phylogenetic Approach to Understand the Evolution of Drug Resistance in Mycobacterium Tuberculosis in Russia, China and South Africa** Pleuni S. Pennings, Marisol Fermin Flores Biology, San Francisco State University

Mycobacterium tuberculosis (Mtb) is the causal agent for a severe bacterial disease that affects the lungs and causes tuberculosis (TB). Due to the increase of drug resistance in Mtb, treating TB effectively has become harder. It is critical to understand the evolution and spread of drug resistance in TB to find ways to prevent drug-resistant infections. In principle, there are two ways in which a patient can get infected with an Mtb strain that is resistant to a given drug: through de novo evolution or via transmission of an already resistant strain. In de novo evolution, the patient is infected with a susceptible strain, which then evolves to become resistant. Alternatively, an already resistant strain is transmitted from another host. It is expected that host-to-host transmission of resistant strains leads to clusters of phylogenetically related strains that share drug resistance mutations. It is not well understood how much resistance is due to transmission. Understanding the importance of transmission for a given drug or country can help guide new policies or interventions to prevent transmission.

Our objective is to determine the relative roles of de novo and transmitted resistance using publicly available data and a phylogenetic approach. The plan is to use a previously established approach in the Pennings lab to (1) create a phylogenetic tree from a publicly available genome sequences and (2) to identify phylogenetic clusters of drug resistant strains (using phytools in R) and (3) compare cluster sizes for resistances to different drugs and for different countries: Russia, China and South Africa. Findings from this study will help us understand the relative roles of de novo evolution and transmission for resistance in Mtb. Understanding Mtb drug resistance in tuberculosis can have a significant impact, as we might be able to provide personalized medicine and uncover ways to mitigate rates of evolution and transmission of resistant Mtb.

839F **The effects of demographic history and linked selection on patterns of genomic diversity in the globally invasive mosquito *Aedes aegypti*** Tyler V Kent, Daniel R Matute, Dan R Schrider University of North Carolina at Chapel Hill

Aedes aegypti, the primary vector of the human arboviruses—dengue, Zika, yellow fever, and chikungunya—was introduced to the Americas beginning with European colonization of South America, and more recently spread throughout the southern United States. Throughout its invasive range, *Ae. aegypti* has faced strong selective pressure from eradication methods, and previous work has suggested there are multiple competing selected haplotypes at loci known to confer insecticide resistance within populations. However, the demographic history of the spread of *Ae. aegypti* within the Americas and the genomic effects of extreme anthropogenic selective pressure are poorly characterized. Here we infer the demographic history of *Ae. aegypti* using previously published resequencing data from 131 samples from Africa and the Americas, including 75 samples from Colombia, Brazil, and the USA. We find evidence for separate introductions throughout the Americas, with strong population structure at fine and coarse scales, variation in the timing of introduction bottlenecks among older and more recently introduced populations, and strong recoveries in population size over short timescales. All introduced populations show an approximately 1.5-fold reduction in neutral diversity, but only minor reductions in the genome-wide efficacy of selection; however, the efficacy of selection is substantially reduced in regions surrounding inferred selective sweeps. Thus, we use a combination of the of the decay of the reduced efficacy of selection around selective sweep signals and forward-in-time population genetics simulations under the inferred demography to estimate the proportion of the genome where linked selection is the primary driver of reduced diversity and efficacy of selection genome-wide in both the native and introduced ranges. This work provides the first detailed population demographic history of *Ae. aegypti* in both its native and introduced range, and more broadly contributes to our understanding of the genomic consequences of the spread of highly invasive species and adaptation to anthropogenic control efforts.

840F **Fat-tailed dispersal facilitate local adaptation in some habitats** Sherif Negm[1], John Novembre[2,3] [1]Human genetics, The University of Chicago, [2]Department of Human Genetics, The University of Chicago, [3]Department of Ecology and Evolution, The University of Chicago

Local adaptation is a central problem in evolutionary biology that arises when individuals in populations exist along an environmental gradient experiencing different selection pressures. This combines two of the major forces that shape the evolution of populations: gene flow and natural selection. Haldane (1930) and Wright (1931) studied the condition when migration overwhelms selection, leading to ‘swamping’ of the locally beneficial allele in the Island-continent model. However, this model assumes discrete population structure while realistically, individuals lie continuously along the environmental gradient. An alternative way to model this is the ‘patch-cline’ model, studied by Slatkin (1973). Here, an allele is favored within a finite length patch in a habitat where individuals can disperse continuously. Under this model, Slatkin (1973) derived a differential equation describing the cline and a ‘characteristic length’ for the patch to show a response to selection while not being overwhelmed by dispersal. However, a main limitation is that this was only confined to short-distance ‘diffusive’ dispersal. Long distance dispersal consequences has been studied for species range expansion (Nichols et Hewitt, 1994) and (Kot et Lewis, 1996). In addition, it has recently been used to model the spread of adaptive alleles (Ralph and Coop, 2010). However, we lack understanding of the effect of fat-tailed dispersal on local adaptation. In this study, we approximate the cline under long distance dispersal by deriving a simple approximate diffusion-convection differential equation for the cline shape under a fat-tailed dispersal kernel. We show that our approximate model matches simulations of the full model under fat-tailed kernel with high accuracy. Counterintuitively, We find that the response to selection in the patch tends to be stronger under fat-tailed dispersal kernels in some habitats, as predicted by the characteristic length. Our results highlight the importance of studying local adaptation under more general dispersal kernels.

841F **Wild bines to beer steins: Exploring the evolutionary genomics of hops domestication** Alexandra McElwee-Adame, Arun Sethuraman Biology, San Diego State University

Humulus lupulus, commonly known as hops, is a perennial crop that natively grows in Asia, Europe, and the United States. For hundreds of years, hops have provided several medicinal, pharmacological, cosmetic, and culinary applications. Of all the industries that the hops plant serves, the largest is the brewing industry. Hops is one of the four key ingredients in

the process of making beer, often added to produce a necessary bitterness from secondary metabolites produced by the lupulin gland in the hop's cones to offset the sweetness of grain. Commercially, there are over 250 different hops cultivars developed worldwide displaying a variety of characteristics in aroma profiles, bittering properties, vigor, and disease tolerance/vulnerability. Previous genomic studies on hops have been dedicated to exploring the genes associated with desirable traits to breeders and brewers alike and how these traits respond to different environmental stimuli, however no studies have explored the evolutionary history of how this plant became domesticated and the genomic consequences of domestication. Our study aims to explore the evolutionary genomics of hops domestication and identify signatures of selection in the domestication process. DNA was extracted from more than 150 cultivars and wild-type samples provided by the USDA Agricultural Research Services. These samples encompassed the total global distribution and purified DNA was sent off for whole-genome sequencing. Assembled genomes will be used to explore the global population structuring, genetic diversity, phylogenetic relationships both between wild and domesticated hops as well as between hops cultivars. Additionally, whole genome sequences will be used to identify regions within cultivar genomes that may display signatures of selection and investigate patterns of gene loss, gain or changes as well as estimate the origin of the hops domestication process. We hope that our findings will provide insight into how hops not only became domesticated but identify the genes responsible for the qualities that are sought after by breeders and brewers.

842F **The effect of long-range linkage disequilibrium on allele frequency dynamics under stabilizing selection** Sherif Negm[1], Carl Veller[2] [1]Department of Human Genetics, The University of Chicago, [2]Department of Ecology and Evolution, The University of Chicago

To understand how selection shapes complex traits in humans, we need to connect population genetic models with data from genome-wide association studies (GWASs). For non-disease traits, a particularly plausible scenario to model is stabilizing selection, which penalizes deviations from an optimal trait value. The basic effect of stabilizing selection on a heritable trait is to reduce the trait's genetic variance. It achieves this in two ways. First, by selecting for compensating combinations of trait-increasing and trait-decreasing alleles, stabilizing selection generates systematic negative linkage disequilibrium between alleles with the same directional effect on the trait (Bulmer 1971). Second, stabilizing selection disfavors rare alleles relative to common ones, causing rare alleles to decrease in frequency in a way that mimics under-dominant selection. The second process, the effect of stabilizing selection on allele frequency dynamics, has recently been shown to be consistent with the genetic architectures of many human traits, as inferred from GWASs (Simons et al. 2018, 2022). Here, we show that these allele frequency dynamics are also influenced by the linkage disequilibrium that stabilizing selection necessarily generates. We exploit a separation of time-scales to quantify this effect, showing that linkage disequilibrium slows down allele frequency changes in a predictable way that depends on measurable parameters of the selection process. Using forward simulations in SLiM, we test our approximation across various genetic architectures, allowing for variation in minor-allele frequencies and effect sizes across loci, and variable genome-wide linkage maps. We explore the implications of the slowdown caused by the Bulmer effect for analyses of the genetic architecture of human traits. We further characterize the effect of this slowdown on population differentiation of traits under stabilizing selection, exploring implications for the "portability" of genomic analyses from one population to another.

843F **Genetic incompatibility increases meiotic nondisjunction in intra-species *C. briggsae* hybrids** Lesly Pereira-Fita[1], Kyle-Asa Lee[1], Joseph Ross[2] [1]California State University, Fresno, [2]Biology, California State University, Fresno

Postzygotic reproductive isolation barriers can arise from genetic divergence between two populations. Such Dobzhansky-Muller interactions involve alleles in different genes that cannot function properly together in the context of an inter-population hybrid genetic background. Because the faithful segregation of sister chromatids during gametogenesis is critical to fertility, and because this process is regulated by many protein complexes, hybrid genetic incompatibilities involving meiosis-related genes could lead to decreased fitness, and potentially thereafter to speciation. It is widely appreciated that the natural frequency of males in androdioecious species of *Caenorhabditis* (e.g. *C. elegans*, which has two sexes: males and self-fertile hermaphrodites) is very low compared to the 1:1 sex ratio that typifies gonochoristic species, which have males and females and are obligately sexually reproducing. *Caenorhabditis* males are genetically XO, and the spontaneous production of males is caused by X chromosome nondisjunction. Thus, male frequency is a proxy for meiotic nondisjunction rates, which are often reported in *Caenorhabditis* as producing about 1 male per 1,000 individuals. *C. briggsae* populations have been useful for investigating genetic incompatibilities, particularly because of their phylogeographic clustering and genetic divergence. We have investigated the existence of genetic incompatibilities between the temperate and tropical circles of latitude clades by establishing multiple crosses between and within clades and populations. We find that many inter-clade crosses produce F1 hybrids with increased meiotic nondisjunction rates compared to within-population control crosses. Thus, spontaneous high incidence of males (*him*) mutations cannot explain the observed data. The results raise the possibility that a dominant genetic incompatibility interferes with meiotic sister chromatid segregation. Although X chromosome nondisjunction is tolerated as a result of dosage compensation, it is possible that nondisjunction of autosomes could simultaneously be producing F2

individuals that have reduced fitness and represent the onset of speciation between the temperate and tropical clades of *C. briggsae*.

844F **Molecular evolution of genes underlying toothed whale acoustic behavior** Leticia Magpali Moura Estevao[1], Elisa Ramos[2], Agnello Picorelli[3], Lucas Freitas[3], Sarvang Dave[4], Mariana Nery[3], Joseph Bielawski[5] [1]Biology, Dalhousie University, [2]Universität Basel, [3]Genética, Evolução, Microbiologia e Imunologia, Universidade Estadual de Campinas, [4]Computer Science, Dalhousie University, [5]Biology; Mathematics and Statistics, Dalhousie University

Acoustic behavior plays a fundamental role in the survival of most animals. For marine mammals, in particular, producing and receiving sounds is the most efficient way of communicating underwater, where vision is often limited. Toothed whales rely on sounds for all aspects of their lives: for instance, they use echolocation to hunt for food, and social sounds like whistles to communicate. Their acoustic behavior depends on specialized biological functions, such as hearing, sound production, cognitive processing and vocal learning. It has been suggested that the environment plays a crucial role in shaping the evolution of toothed whale sounds, however, this hypothesis has yet to be addressed under a phylogenetic framework. Furthermore, little is known about the genes underlying toothed whale acoustics and how they evolved in species that colonized different habitats. To address these questions, we analyzed a set of candidate genes potentially involved in acoustic behavior across 37 species of toothed whales. We used a combination of statistical models to compare the evolutionary rates of these genes across riverine, coastal, and oceanic toothed whales. For the hearing genes CDH23, TMC1 and SLC26A5, we found significant rate variation among toothed whales from distinct habitats, in combination with strong evidence of positive Darwinian selection. We also uncovered positively selected sites located near functional protein regions, and statistically associated with radical amino acid changes or habitat differences. Overall, coastal and riverine lineages experienced accelerated evolution compared to oceanic species. Lineages under positive selection, or enriched for positively selected sites, were either from riverine/coastal shallow waters, or deep diving oceanic species. Coastal dolphins under positive selection have recently diverged from riverine sister species, suggesting that historical selective pressures could have triggered rapid adaptation to the coastal environment. Finally, we uncovered potentially convergent substitutions among species that share similar sonars, such as river dolphins and Narrow-Band High-Frequency echolocators. Our findings corroborate and extend previous suggestions that specific environments imposed unique selective pressures on toothed whale echolocation genes and phenotypes. Our study suggests multiple pathways for the evolution of the toothed whale sonar, and contributes to unraveling the molecular basis of environmental adaptation. Future work will focus on a network of genes functionally connected to sound production, hearing, processing and vocal learning. Joint analysis of phenotype-genotype within the context of a functional network could illuminate new genetic systems associated with acoustic behavior in cetaceans, and further improve our understanding of how toothed whale sounds evolved in different habitats.

845F **The Impact of Treatment Timing on the Probability of Cure in Two-strike Therapy Protocols** Amjad Dabi[1], Daniel Schrider[2] [1]University of North Carolina at Chapel Hill, [2]Bioinformatics and Computational Biology, UNC Chapel Hill

The emergence of resistance to therapeutic agents poses a difficult challenge to treatments involving clonal populations such as bacteria or cancer cells. When resistance to first-line treatments becomes evident, a standard practice is to switch to second-line treatments. However, it is not yet clear what the optimal timing of the second-line treatments is, and whether administering such treatments before evidence of resistance is present would yield better outcomes. In this study, we use forward-in-time genetic simulations, adapting evolutionary rescue models to simulate the expansion and treatment of a clonal population in a two-strike therapy regime and investigate the impact of various parameters, including the pre-treatment population size, mutation rate, treatment efficacy, and the fitness advantage of resistance. The simulated population expands exponentially from a single cell until reaching a specified size, after which two treatments are applied serially. Treatment efficacy is modeled as the per-generation death probability of cells during treatment, and each treatment has an associated rate of mutations that confer resistance to that treatment by lowering the per-generation death probability of cells harboring them. Consistent with previous work in evolutionary rescue, we find that increasing the mutation rate or pre-treatment population size increases the likelihood of rescue, while increasing treatment efficacy results in the opposite effect. Given the clinical importance of treatment timing, we also explore its effect on outcomes. Our results indicate that the timing of the second strike is an

important factor and that in some cases, second strikes that precede the emergence of resistance to the first strike are more effective at preventing the emergence of resistance and subsequent evolutionary rescue.

846F **Pervasive paternal mitochondrial transmission in *C. briggsae* hybrids** Joshua Proctor[1], Kevin Helwick[1], Joseph Ross[2] [1]California State University, Fresno, [2]Biology, California State University, Fresno

Strictly maternal mitochondrial transmission to offspring might have evolved to prevent heteroplasmy, which is the presence in a cell of mitochondria with different mitochondrial genotypes (mitotypes). Paternal mitochondrial transmission (PMT), though an unexpected phenomenon, has been occasionally observed in several species in crosses between genetically diverse parents, including in *Caenorhabditis briggsae*. However, the molecular mechanism by which PMT occurs is unclear. Hybrids of genetically divergent parents might experience PMT as a result of genetic incompatibilities. Thus, we hypothesized that PMT frequency is directly related to the amount of genetic divergence between parents. Essential resources that supported this work include the availability of multiple *C. briggsae* populations at the CGC as well as known phylogeographical relationships between them. We first created restriction fragment length polymorphism (RFLP) PCR assays that can distinguish the mitotypes of various *C. briggsae* populations. We then created multiple cytoplasmic-nuclear hybrid (cybrid) lines: some from crosses between more genetically similar populations (e.g. within-clade tropical x tropical), and others from crosses of populations from different clades (e.g. between-clade temperate x tropical). Each line was created by a five-generation serial paternal backcross followed by five generations of selfing. DNA from each line was purified and genotyped to detect heteroplasmy. PMT, evident as heteroplasmy, was observed in the majority of lines, regardless of whether a within-clade or a between-clade cross. There was no significant difference in the frequency, which suggests that genetic divergence between populations does not directly influence PMT rates. One interpretation of these results is that *C. briggsae* might be inherently unable to prevent PMT. Future work should monitor mitochondrial transmission dynamics at fertilization (both in self-fertilization and also in crosses between genetically identical males and hermaphrodites), which cannot be accomplished using our PCR-based approach. The prevalence of PMT in *C. briggsae* crosses motivates further work to investigate the genetic and molecular basis of mitochondrial transmission regulation.

847F **Signatures of selective sweeps in continuous-space populations** Meera Chotai, April Wei, Philipp W Messer Computational Biology, Cornell University

Selective sweeps describe the process by which an initially rare advantageous mutation increases in frequency in a population, thereby removing genetic variation in its genomic vicinity. Hard sweeps originate from a single haplotype, while soft sweeps describe scenarios where multiple haplotypes carrying the beneficial allele spread at the same time. The signatures of hard and soft sweeps have been studied primarily under panmictic models, but natural populations are often structured spatially, which could affect sweep signatures. To investigate this effect, we simulated selective sweeps in populations inhabiting two-dimensional continuous space, where the dispersal range of offspring from their parents can be varied from limited to extensive, at which point it is equivalent to a panmictic population. We found that advantageous mutations spread more slowly in a population with limited dispersal as compared to a panmictic population, leading to a lower fixation probability. At the same time, recombination is less effective at breaking up genetic linkage in populations with limited dispersal. Together, these effects result in selective sweeps that extend over similar genomic sizes across dispersal rates. However, hard sweeps in the low-dispersal regime tend to have higher haplotype heterozygosity, bearing a resemblance to soft sweeps. These results highlight the need for better accounting of spatial population structure when making inferences about selective sweeps.

848F **A genealogical interpretation of LD at a small locus** Mariadaria K Ianni-Ravn[1], John Novembre[2] [1]Human Genetics, University of Chicago, [2]University of Chicago

When mutations occur in close proximity to each other, they are correlated until recombination breaks down the linkage disequilibrium (LD) between them. The signed LD between pairs of mutations describes the probability of them being found on the same or different haplotypes more or less often than expected if they were unlinked. Although in principle mutations can exhibit positive or negative epistatic fitness interactions (Crow 2010), LD decreases the overall efficacy of selection and increases the probability of deleterious alleles fixing in a population - a phenomenon known as ‘selective interference’ (Hill and Robertson, 1966). Although the emergence of LD has been studied extensively (for example, McVean, 2002; Ragsdale and Gravel, 2019; Good, 2022), most focus has been on modelling patterns over many generations due to recombination; there has been less theoretical work on the dynamics within a small locus over short timescales, before recombination can cause the correlation structure to decay. However, without an understanding of this, it is difficult to interpret patterns of variation and the allelic structure at genomic regions in the kilobase scale.

In this study, we focus on the signed LD resulting from “genealogical correlation” - the linkage implied by the relative

placement of mutations on the genealogy describing a locus. This allows us to understand how often pairs of mutations should be in positive or negative LD, given that they occur in close proximity to each other. In short, mutations located “downstream” of each other on a genealogy exhibit positive correlation, while those on opposing sides of the genealogy display negative correlation. We derive the probabilities of these relationships under neutrality, and use a simulation approach to show that they can be affected by forces which distort the genealogical process - such as non-equilibrium demography and selection. Finally, we apply our reasoning to interpret patterns of signed LD at specific genomic loci in humans, such as CFTR and MHC. These results help us to understand the emergence of dominance, and emphasize that LD is not a static feature, but rather is a dynamic force which both affects and is affected by population genetic processes.

849F **Investigation of putative parthenogenesis in the vulnerable Jamaican Boa (*Chilabothrus subflavus*)** David Graber, Kyle Mara, Kimberly Delaney Biology, University of Southern Indiana

Facultative parthenogenesis (FP) has been demonstrated in numerous animal taxa such as: sharks, varanid lizards, birds, and snakes. There are multiple mechanisms by which a female of a dioicous species can produce offspring asexually without genetic material from a male mate including apomixis, central fusion automixis, and terminal fusion automixis. While FP via terminal fusion automixis has been demonstrated in multiple boa species, the Jamaican boa (*Chilabothrus subflavus*) has not previously demonstrated FP. An eight-year-old captive female Jamaican boa, housed in a private collection, gave birth to two living and multiple nonliving offspring after a period of isolation lasting her entire adult life. This female was separated from all other individuals from at least six months of age and any previous contact with males came from her own clutch. We isolated DNA samples from the female, her offspring, and a male clutch mate. Using short tandem repeat analysis of multiple nuclear microsatellite loci previously described in this species, we will present genetic data from these samples which will help elucidate the origin of this reproduction event. Neither sperm storage of this duration nor parthenogenesis have been previously documented in this species. While a sperm storage event is theoretically possible, it is highly unlikely as the last potential contact the female had with a male mate would have been just after birth. We believe this reproduction event represents the first documented case of parthenogenesis in this species. Events of this nature may have significant implications for the genetic diversity of the endemic population of this vulnerable species and the captive breeding programs in place to preserve the species.

850F **When does adaptation arise from a polygenic response versus few large effect changes** William Milligan[1], Laura Hayward[2], Guy Sella[1] [1]Biological Sciences, Columbia University, [2]Institute of Science and Technology Austria

When does adaptation arise from a polygenic response versus few large effect changes? This question traces back to early debates sorounding Darwin’s “Origin of species” and remains wide open today. Strong but indirect evidence suggest that polygenic adaptation should be ubiquitous, but demonstrations have proven elusive. In turn, there are hundreds of examples of large effect adaptations in many species, but it remains unclear whether they represent a common mode of adaptation in any given species. Translating these disparate evidence into general answers is complicated by the fact that the approaches to studying adaptation are designed to answer different questions and differ in their limitations and biases. We propose that a productive way forawrd starts with reframing the question in terms of traits and asking: how does the genetic basis of adaptation depend on the properties of genetic variation in a trait and on changes in selection pressures acting on it. We study this question in a simple yet highly relevant setting. We consider a quantitative trait subject to stabilizing selection around an optimal value, and model the response to selection when a population at MSDB experiences a sudden shift in this optimal value. We characterize the adaptive response in this setting both quantitatively and qualitatively, specifically, we delimit how the adaptive contributions of large-effect and polygenic changes depend on a trait’s genetics and ecology. We discuss other salient factors that affect adaptation, how the theory we present and its future development could help us to understand when different modes of adaptation are expected, and how such theory can provide a framework within which to interpret the diverse and growing body of evidence about the genetic basis of adaptation.

851F **Genetic variation in prey size and movement interacts to affect predation risk in *Paramecium caudatum*** Kyle Coblentz, Kristi Montooth, John DeLong School of Biological Sciences, University of Nebraska-Lincoln

Species’ traits play a key role in determining the outcomes of predator-prey interactions with consequences for population dynamics, food webs, and trait evolution. Few studies have evaluated the heritability of traits related to predation risk and their potential genetic correlations to predict how selection will shape predator-avoidance traits of prey. Moreover, given the pervasive effects of temperature on the development of traits related to size and movement in ectotherms and the fact that temperature also affects how predators and prey interact, it is likely that prey trait evolution to temperature will be impacted by the presence of predators. We are using the freshwater ciliate *Paramecium caudatum* as a model system to investigate how predators shape prey trait evolution and how this evolution impacts predator-prey dynamics in the context of a warming thermal environment. We will present data demonstrating significant genetic variation for morphology and movement behavior in outcrossed, clonal lines of *P. caudatum* from Nebraska ponds. Genetic variation in prey traits significantly affected

fitness, as it altered *Paramecium* susceptibility to predation by the copepod *Macrocyclops albidus* also collected from Nebraska ponds. The proportion of *Paramecium* consumed by copepods was positively associated with *Paramecium* body size and greater velocity as one might expect from interspecific relationships. However, we also found evidence of an interaction such that greater velocities led to greater predation for large body-sized *Paramecium* but did not alter predation risk for smaller body-sized *Paramecium*. In contrast to what has been observed in interspecific allometric relationships, body size and movement speed did not positively covary across clonal lines. These patterns of predation risk and heritable variation in the traits of *Paramecium* together suggest that copepod predation likely acts as a selective force that could operate independently on movement speed and body size and operate most strongly on large, fast *Paramecium*. We are testing these predictions using this pool of genetic variation in a long-term evolution experiment that evolves *Paramecium* to warmer and colder thermal environments in the presence or absence of predators.

852F **Investigating the effects of voltinism polymorphism on the rate of molecular evolution in the European corn borer (*Ostrinia nubilalis)*** Alejandro Calderon, Erik Dopman, Lawrence Uricchio Biology, Tufts University

Adaptation may be a crucially important driver of population outcomes for species experiencing variable environments. Many terrestrial arthropod species have intraspecific variation of voltinism (number of generations per year) due to seasonal variation, and population genetics theory suggests that generation time may be a determinant of the rate of molecular evolution. However, the adaptive significance of polymorphic voltinism has not been fully elucidated, despite of genomic characterization of some species' circannual variation. Species with polymorphic voltinism may provide a unique view of molecular evolution because intraspecific variation in generation time may have detectable effects on patterns of polymorphism and divergence. In this study, we investigate the role of polymorphic voltinism in shaping the rate of molecular evolution in *Ostrinia nubilalis*, the European corn borer (ECB) moth. Prior studies revealed that two clock genes, period (*per*) and pigment-dispersing receptor factor (*Pdfr*) might be the genomic determinants of circannual rhythm in ECB. Our project leverages pool seq data from 14 populations that vary in voltinism, allowing us to explore the relationship between generation time and patterns of polymorphism within ECB. We use a molecular evolution model that incorporates demographic and selective effects to investigate how generation time variation may affect variation in voltinism loci (*per* and *Pdfr*) as well as the background genome.

853F **Constraining models of dominance of deleterious mutations in the human genome** Christopher D. Kyriazis, Maria Izabel Cavassim, Kirk E Lohmueller UCLA

Dominance is a fundamental parameter in genetics, determining the mode of natural selection on deleterious and beneficial mutations, the relationship between population size and deleterious genetic variation, and the severity of inbreeding depression in a population. Despite this importance, very little is known about dominance, particularly in humans or other non-model organisms. A key reason for this lack of information about dominance is that it is extremely challenging to disentangle the selection coefficient (*s*) of a mutation from its dominance coefficient (*h*). Here, we explore dominance and selection parameters in humans by fitting models to the site frequency spectrum (SFS) for nonsynonymous mutations. We employ the diffusion-based approximation of allele frequency change in the Poisson Random Field framework. We find that numerous selection and dominance models can fit the data, so long as mean h>~0.15 and weakly deleterious mutations ($10^{-4}<s<10^{-3}$) are not too recessive. Moreover, we also find that theoretically-predicted models with a relationship between *h* and *s*, where deleterious mutations tend to be more recessive, can also fit the data well. Through model averaging, we estimate a mean *h* for nonsynonymous mutations of ~0.3, though with considerable uncertainty in *h* for individual selection coefficient bins. For models that fit the data, the inferred distributions of fitness effects (DFE) are relatively robust to different values of *h*, validating previous estimates of the DFE. Finally, we use our estimated dominance and selection parameters to inform simulations revisiting the question of whether the out-of-Africa bottleneck has led to differences in genetic load between African and non-African human populations. This analysis suggests that, under realistic dominance models with partially recessive mutations, genetic load is slightly elevated in non-African human populations, a result that cannot be detected by counting the number of derived deleterious alleles. Moreover, these results also suggest that models of partially recessive nonsynonymous mutations can also explain the observed severity of inbreeding depression in humans. Our work represents a comprehensive assessment of dominance in humans, with implications for parameterizing models of deleterious variation in humans and other mammalian species.

854F **Whole genome sequence data elucidates signatures of cattle domestication and global bovid migration patterns** John L Miraszek[1], Robert Schnabel[2], Jared Decker[2] [1]Genetics Area Program, University of Missouri, [2]University of Missouri

Domesticated cattle and its wild bovid relatives are a clade with rich evolutionary history intrinsically linked with anthropogenic forces. Of particular interest to the fields of population genetics, anthropology and evolutionary biology, is

the process of domestication. While directional-selection on the complex of traits encompassed in 'domestication syndrome' has been previously identified with soft-sweeps, it remains unclear whether this polygenic selection was a gradual process, or an initial confluence of related molecular events allowed this compounded phenotype to arise relatively quickly, as in the neurocristopathic hypothesis. Additionally, the classical view that domestication resulted from a bottleneck event with persistent isolation has been questioned due to the high pervasiveness of gene flow between wild and non-wild animals. Considering that within the Bos clade, animals from traditionally wild populations such as the yak and gaur were subsequently domesticated through hybridizing with the previously domesticated taurine and indicine cattle, there is ample opportunity to investigate domestication as emergent through distinct mechanisms within this genus. Past efforts in cattle and other domestic mammalian species either lacked samples with enough diversity, quality or overall size to uncover many of the genetic signatures of selection or accurately map dynamics of migration and admixture on a global scale. We use 1810 resequenced genomes from over 200 breeds of wild and domestic animals from the genus Bos and utilizing the latest demographic methods which proved highly effective and scalable for large sample sizes in human studies, estimate a genealogy for domestic cattle across thousands of individuals of diverse origins, the relative time scale of coalescent events, ancestral allele frequencies, and mutation rates following introgression. This will allow us to detect evolutionary forces that were present during domestication, such as selective pressure on polygenic traits, and the impacts of wild species interbreeding to better elucidate the genetics behind domestication in the context of human history and biogeographical space and time.

855F **Associating Genes with Diet through Convergent Evolution**

Michael Tene[1], Mathew Pollard[2], Emily Puckett[2], Wynn Meyer[1] [1]Biology, Lehigh University, [2]University of Memphis

Convergent evolution is when multiple species evolve the same trait independently. By examining the genes with common patterns of relative evolutionary rate across these independent replicates of evolution, we can detect genes which are associated with the evolution of the trait. Using this method, we have identified several genes and pathways associated with diet evolution in mammals. Our work is focused on the evolution of carnivory, and we have additionally examined the evolution of omnivory and herbivory.

We have examined different definitions of carnivory: carnivory as binary opposed to herbivory, carnivory as a continuous scale based on the percentage of diet composed of vertebrates, and carnivory as one of multiple diet categories. Using differences in which genes are associated with each definition of carnivory, we provide insight into which aspects of carnivory may drive evolutionary rate changes.

To perform this analysis, we must determine the phenotype of all of the branches in the phylogenetic tree. Particularly relevant are branches corresponding to the ancestors of extant species, which cannot be directly measured. We can estimate ancestral states either through ancestral state reconstruction, or through referencing the fossil record. Additionally, we can only assign ancestral branches phenotypes included in the analysis, meaning some branches may be incorrectly assigned, particularly by phylogenetic inference. We have examined the effect of ancestral branch phenotype assignment by using different methods of ancestral state inference and inclusion of different non-carnivory phenotypes in the analysis.

We have found metabolic genes to be evolving at different rates in carnivores and herbivores. Amino acid and lipid metabolism evolve more slowly in carnivores than herbivores. We find genes expressed in the brain, liver, kidney, and thyroid to evolve more slowly in carnivores than herbivores. The reduced evolutionary rate of amino acid metabolism and the above tissues suggests they have been subject to increased purifying selection in carnivores, thus implying greater importance in carnivores. We have also found that various muscle function genes and muscle tissues evolve more rapidly in carnivores than herbivores, implying either increased conservation in herbivores or positive selection in carnivores. In the future, we hope to look for associations in non-coding element evolution, and assess the three dimensional structural effects of the residues driving the different rates of coding sequence evolution that we detect here.

856F **Inferring the distribution of fitness effects from genetic variation with convolutional neural network** Linh Tran, Ryan Gutenkunst University of Arizona

The distribution of fitness effects (DFE) of new mutations quantifies the input of mutations with certain selective effects (deleterious, neutral, advantageous) into natural populations and is fundamental to evolution by natural selection. The DFE is particularly important for understanding quantitative traits, such as the genetic architecture of complex diseases. All previous studies that estimated the DFE from genetic variation data used summary statistics describing some aspects of the data, such as the allele frequency spectrum and/or linkage disequilibrium statistics. While powerful, these existing approaches either neglect substantial information from the data not included in the chosen summary statistics or demand careful curation of the appropriate set of summary statistics.

Here we introduce the first deep learning approach for DFE inference using convolutional neural network (CNN) and a novel representation of genetic variation data. Single nucleotide polymorphism (SNP) alignment is represented by a three-dimensional tensor in which each layer corresponds to a functional class, such as synonymous and nonsynonymous coding variants. With this representation, our approach allows direct processing of genetic variation data in which the CNN implicitly learns the most informative features in the data, bypassing summary statistics selection and assumption.

We trained and validated the CNN using data simulated under varied gamma DFE distributions and human demographic histories. We found that the trained CNN achieves better inference accuracy on simulated data than a commonly used summary-statistics-based DFE inference method. Our result demonstrates that deep learning is a promising and powerful approach for fully harnessing the information available in modern genomic data sets to infer the DFE with higher precision.

857F **Inter-chromosomal linkage disequilibrium in rice populations** Anthony Greenberg Bayesic Research

Levels of linkage disequilibrium (LD) among unlinked sites reflect the interplay of population genetic forces shaping the history of a species. For example, theory suggests that LD is more sensitive to population stratification than sequence divergence at individual sites. However, enumerating pairs of unlinked correlated loci normally requires the examination of all pairwise relationships in a genome. This task becomes computationally prohibitive with modern data sets comprising millions of polymorphisms. I have developed an approximate hash table based method to identify groups of loci in LD across the whole genome that does not require computing associations between uncorrelated pairs. I used this method to study LD among unlinked sites in populations of Asian domesticated rice, *Oryza sativa*. This species consists of two well-defined varietal groups, *INDCIA* and *JAPONICA*, with further shallower subpopulation division into *indica*, *aus*, and tropical and temperate *japonica*. These populations vary in the extent of local linkage disequilibrium, with *indica* the lowest and *temperate japonica* the highest. *O. sativa* is thus a good testing ground for the application of my method, taking advantage of the rich genomic resources including whole-genome sequence of 3000 accessions and a well-annotated reference. I will describe the analyses and several permutation tests enabled by the fast whole-genome LD estimation. These tests shed new light on the sources of among-population variation in LD among unlinked sites.

858F **Non-coding Variant Discovery with Evolutionarily-Informed Probabilistic Machine Learning Models** Courtney Shearer, Evan Cofer, Rose Orenbuch Harvard

Non-coding regions make up the majority of the genome and house thousands of variants associated with human disease. Unfortunately, when non-coding variants are identified in genomics studies, we are unable to identify whether these variants are actually causal or simply in linkage disequilibrium with the causal variants. Existing methods to prioritize non-coding variants generally rely on training convolutional neural networks on epigenetic data, and fail to identify variants that may function in biological contexts for which no such data exist. Clearly, there is a need for models that can uncover pathogenic non-coding variants associated with heritable traits and diseases irrespective of the available high-throughput regulatory sequencing studies (e.g. ChIP-seq). Here, we borrow modeling approaches from evolutionarily-informed generative models for proteins, and apply them to non-coding regions such as promoters to accurately predict the pathogenicity of non-coding variants. We outperform existing methods, and demonstrate our model's utility for prioritizing both rare and common novel disease-associated variants for a range of heritable diseases. Finally, we establish a set of public and rigorous benchmarks for future non-coding variant prioritization tools to accelerate community efforts in this area.

859F **The contribution of transposable element insertions to genetic diversity and recent adaptation in *Aedes aegypti* populations** Gabriela Valente-Almeida[1], Austin Daigle[2], Daniel Schrider[2] [1]University of North Carolina Chapel Hill, [2]Genetics, University of North Carolina Chapel Hill

Transposable elements (TEs) are mobile DNA sequences that can replicate themselves and be inserted in different genome regions. These are present in almost all organisms and comprise a significant part of the genome. TE insertions often have deleterious effects, as they may disrupt genes or regulator elements. Because of their repetitive nature, identifying these structural variants from short-read sequencing technology is a major challenge, and therefore much is still unknown about the population genetic dynamics of TEs. Here we characterize the diversity of TE insertions within populations of Aedes aegypti, an important vector of pathogens that cause multiple tropical diseases, such as Dengue, Zika, Chikungunya, and Yellow Fever. Cataloging genetic diversity within this species is of great interest, and surveying TE composition is an important component of this effort, as such mutations have been shown to facilitate insecticide resistance in other species. Our aim in this project is to gain a deeper understanding of the composition and potential effects of transposable elements in Aedes aegypti populations. To this end, we detected TE insertions from 110 samples from six different locations spanning Africa, North America, and South America, and sought to identify the evolutionary effects of these TEs on these different populations. We were able to accurately identify both reference and non-reference TEs in this dataset and therefore examine patterns of diversity within

and between populations. In total, 371134 insertions were identified in all our reference TE datasets, and 282034 insertions were identified in all our non-reference TE datasets. We found that, as expected, TEs were confined to lower allele frequencies than intergenic SNPs, consistent with negative selection Lastly, we compare the frequencies of TEs across geographic locations, discovering several candidates for local adaptation in this important vector species that is rapidly adapting to anthropogenic selective pressures.

860F **Non-adaptive mechanisms of metabolic evolution of *Escherichia coli* in a complex environment** Wei-Chin Ho[1,2], Lily King[2], Ryan Stikeleather[2], Michael Lynch[2] [1]University of Texas at Tyler, [2]Arizona State University

The capacities to utilize carbon sources are critical for microbes to propagate and mediate essential ecological functions in ecosystems. Although the existing carbon sources are thus expected to largely dictate the microbial evolution of metabolic competencies, the observation can sometimes be puzzling. For example, microorganisms adapting to one carbon source can simultaneously lose and gain the abilities to grow in other carbon sources, suggesting the involvement of neutral accumulation of loss-of-function mutations and/or pleiotropic adaptations. However, the relative contributions of these forces remain unclear, especially for complex environments with multiple carbon sources. To address this gap, we surveyed 32 *Escherichia coli* populations that had experienced 900 days of experimental evolution under two mutation rates and two transfer sizes in lysogenic broth, which comprises a mixture of amino acids. Specifically, their metabolic competencies were evaluated by growth in single amino acids. As a result, we found cases of increased growth in glutamic acid, histidine, or proline as well as decreased growth in alanine. More evolution of decreased growth is related to higher mutation rates but not higher adaptive efficacies owing to higher transfer sizes. This finding suggests that loss-of-function mutations are critical for metabolic-competency evolution. Additionally, we identified a substitution in proQ that enhanced growth in alanine. Interestingly, this substitution is not beneficial in lysogenic broth, highlighting the role of non-adaptive forces in novel metabolic competencies. Overall, our results identify pivotal molecular and evolutionary mechanisms influencing metabolic evolution, demonstrating the possibility of niche partitioning and specialist evolution even in complex environments.

861F **The cellular evolution underlying variation in innate social behavior across *Peromyscus* deer mice** Jenny Chen, Chris Kirby, Phoebe Richardson, Hopi Hoekstra, Sean Eddy Harvard University

Understanding how genes encode for something as complex as social behavior is an open question in biology. Variation in innate social behaviors across species offers an opportunity to use a comparative approach to identify the genetic building blocks of behavioral traits. Here, we investigate two closely-related species of *Peromyscus, P. maniculatus and P. polionotus,* that lie on opposite ends of the promiscuity-monogamy spectrum and exhibit vast differences in mating and parentin behavior. We focus on the hypothalamic medial preoptic area (MPOA), a region known for its role in social behavior, and asked how differences in the cellular and molecular composition of this region contribute to the differences in social behavior of these two species. We perform single nuclei RNA-sequencing in 6 females and 6 males of each species. Across species, we find several differentially abundant cell types, two of which have been previously implicated in parental care behavior. Across sexes, we found significantly fewer sex-biased genes in the monogamous *P. polionotus* compared to *P. maniculatus*, consistent with the reduction of sexual dimorphism observed in monogamous species. Both differentially abundant cell types and sex-biased genes are significantly enriched to be neuropeptidergic. Together, we find evolution of social behaviors involve changes in both cell abundance and sex-biased expression, with neuropeptide regulation likely playing a crucial role

862F **balselr - Balancing Selection Tests in R** Daphne Hansell[1], Barbara Domingues Bitarello[2] [1]Bryn Mawr College, [2]Biology, Bryn Mawr College

Long-term balancing selection (LTBS) is vital for population adaptability and resilience. LTBS includes mechanisms such as heterozygote advantage, negative frequency dependency selection, and fluctuating selection, all of which maintain polymorphisms segregating in populations. We previously introduced the Non-central Deviation (NCD) statistic as a statistically powerful and fast method to identify LTBS. Widespread adoption of NCD and several other LTBS methods developed since has been limited due to specialized knowledge requirements and implementation inaccuracies. Our package described here bridges this gap by providing an accessible interface, enabling researchers to leverage single-locus and genomic data effortlessly, even with basic R proficiency. We name this package balselr — balancing selection in R. balselr is a comprehensive R package designed to detect LTBS using the NCD statistics as initially described. balselr features include: a) preparation of input files from popular data formats, such as VCF files for SNP data (e.g. functions read_vcf() and parse_vcf()); b) implementation of NCD1 and NCD2 calculations (ncd1() and ncd2() functions, respectively); help pages and tutorials to further aid researchers in understanding LTBS prevalence and implications in population and evolutionary genetics. This dedicated package will make it easier for individuals with basic proficiency in R to run the NCD method effectively, allowing wider adoption of NCD and advancing understanding of balancing selection's evolutionary significance across organisms. We implemented comprehensive automated package tests to ensure, for example, that if a function code is changed, the output

stays the same, that functions produce the correct type of object, and to compare an expected calculation to the functions' output. Ongoing and future work include: implementing more tests for the existing functions; including other LTBS methods beyond NCD that are currently not implemented in R (e.g. BetaScan and BalLeRMix)); integrating the balselr and slendr (Population Genetic Simulations in R) packages and providing tutorial materials on how to properly assess statistical power for different organisms with forward simulations. In brief, the current package and the planned updates will make it easier for researchers with minimal programming experience to use NCD and other LTBS methods on their data. The development version is available at https://github.com/bitarellolab/balselr.

863F **Adaptive significance of amphicarpy as a bet-hedging strategy in American hog-peanut** Neal Yin, Maya Weissman, David Peede, Daniel Weinreich Ecology, Evolution, and Organismal Biology, Brown University

Environmental instabilities call for organisms to develop adaptations for long-term survival. Bet-hedging is one such strategy aimed at reducing the risk of extinction in changing environments, where a population sacrifices its short-term population-wide mean fitness in order to minimize long-term variance fitness in the long run. One potential bet-hedging strategy in plants is amphicarpy, a reproductive strategy where individuals produce two morphologically distinct types of seeds: subterranean seeds and aerial seeds. Subterranean seeds are produced asexually and are large, robust, and fecund when mature. On the other hand, aerial seeds are less fit than their underground counterparts but are more abundant, occasionally result from sexual reproduction, and experience delayed seed germination. It has been hypothesized that amphicarpy is a bet-hedging strategy, however rigorous tests of whether or not amphicarpy is adaptive and meets the criteria for bet-hedging are lacking. We evaluate amphicarpy as a form of bet-hedging in the American hog-peanut (Amphicarpaea bracteata), an amphicarpic annual native to a wide range of environments. We simulated the evolution of amphicarpy using stochastic Wright-Fisher models incorporating other annual plant reproductive behavior, including seed-banking (delayed seed-germination) and predation. Empirical parameters were drawn from prior in-field studies and approximately 200 American hog-peanut plants grown in the university greenhouse. We find that amphicarpy in the hog-peanut is a bet-hedging strategy, but seed banking alone does not explain the observed amphicarpic ratio. Rather, predation, biological constraints, and other fitness effects are necessary to capture the adaptive significance of amphicarpy.

864F **Scaling the fitness effects of mutations with respect to differentially adapted *Arabidopsis thaliana* accessions under natural conditions** Frank Stearns[1], Juannan Zhou[2], Charles Fenster[3] [1]Biology, Stevenson University, [2]Biology, University of Florida, [3]Biology and Microbiology, South Dakota State University

Mutations are the ultimate source of genetic variation for natural selection to act upon. A major question in evolutionary biology is the extent to which new mutations can generate genetic variation under natural conditions to permit adaptive evolution over ecological time scales. Here we collected fitness data for chemically induced (ethylmethane sulfonate, EMS) mutant lines descended from two *Arabidopsis thaliana* ecotypes that show differential adaptation to the local environment of our common garden plot. Using a novel nonparametric Bayesian statistical approach, we found that both ecotypes accumulated substantial proportions of beneficial mutations. The poorly adapted ecotype showed higher variance in the fitness effect of mutations than the well-adapted ecotype. Furthermore, we predict that it takes less than 4000 generations for the fitness space of the two ecotypes to overlap through mutation accumulation, and that a single founder, through mutation accumulation, is able to achieve the species-wide genetic variation in less than 10,000 generations. Our results provide evidence for relatively rapid local adaptation of *Arabidopsis thaliana* in natural conditions through new mutations, as well as the utility of nonparametric Bayesian method for modeling the distribution of fitness effects for field-collected data.

865F **Identifying candidate loci for hybrid seed inviability within *Mimulus* interspecific crosses** Natalie M. Gonzalez Genetics, University of Georgia

A common barrier to plant hybridization is the failed development of endosperm which leads to hybrid seed inviability. Despite its potential importance in angiosperm speciation, the genetic basis of hybrid seed failure remains almost completely unknown. Within the genus *Mimulus*, hybrid seed inviability has been described in several interspecific crosses including those of the recently diverged species *M. tilingii* and *M. caespitosa*. In this species pair, F1 hybrid seeds in one direction of the cross (*M. tilingii* as the pollen parent) are almost always inviable, whereas F1 seeds from the reciprocal cross are normal. Here we use a powerful crossing design, generating reciprocal backcross progeny to both species, to genetically map loci involved in *M. caespitosa-M. tilingii* F1 seed lethality. We have generated RADseq genotypes for each of the four backcross populations and are now characterizing genome-wide patterns of transmission ratio distortion to identify regions associated with seed failure. Paired with our recent characterization of genomic imprinting in this system, this dataset is an important step toward discovering the molecular genetic basis of *Mimulus* hybrid seed inviability.

866F **Genome architecture influences copy number variant dynamics in adapting populations** Julie Chuong, Ina Suresh,

Julia Matthews, Titir De, Grace Avecilla, Farah Abdul-Rahman, David Gresham Biology, New York University

DNA mutations are the raw material for genetic variation, evolution, and biodiversity. Copy number variants (CNVs)— gains and losses of genomic sequences—are a pervasive class of mutation that frequently underlie rapid adaptation. Despite their central role in evolution, it is unknown how genomic features influence CNV formation rate, location, size, and dynamics in evolving populations. Here we investigate the effect of proximate sequence elements at the *GAP1* locus, which encodes the general amino acid permease gene, on CNV dynamics in Saccharomyces cerevisiae.

The *GAP1* gene has a unique genomic architecture consisting of two flanking Ty1 long terminal repeats (LTRs) and an autonomously replicating sequence (ARS), which are thought to facilitate CNV formation at high rates. To test the effects of local genome architecture on *GAP1* CNV dynamics, we engineered three strains: any one lacking either an ARS, two LTRs, or all three elements. Then we experimentally evolved populations in glutamine-limited chemostats, a selective environment in which we have previously observed *de novo GAP1* CNVs. We detected *GAP1* CNVs in evolving populations over 200 generations using a fluorescent CNV reporter system.

Our results recapitulate previous findings that *de novo GAP1* CNVs are repeatedly formed and selected for early during adaptive evolution before undergoing more complex dynamics. Surprisingly, we found the local architecture elements were not required for *GAP1* CNV formation, highlighting the pervasiveness of CNVs during adaptive evolution. Whole-genome-sequencing of isolated CNV clones and subsequent inference of CNV mechanisms showed that in the absence of local features, CNVs form using distal ones. This study shows that local and distal DNA features facilitate CNV formation at high rates thereby fueling rapid adaptation in selective environments.

867F **The histone deacetylase Hos2 regulates protein expression noise by modulating protein translation machinery** Wei-Han Lin, Jun-Yi Leu Institute of Molecular Biology, Academia Sinica, Taiwan

Non-genetic variations derived from expression noise at the transcript or protein levels can lead to cell-to-cell heterogeneity within an isogenic population. While cells have developed strategies to reduce noise in some cellular functions, this heterogeneity can also facilitate different levels of regulation and provide evolutionary benefits in fluctuating environments. Although several general characteristics of expression noise have been revealed, detailed molecular pathways underlying noise regulation remain elusive. Here, we established a dual-noise reporter system in *Saccharomyces cerevisiae* and performed experimental evolution to search for mutations that increase the expression noise. By analyzing evolved cells using bulk segregant analysis coupled with whole-genome sequencing, we identified histone deacetylase, Hos2, as a negative noise regulator. *hos2* mutants down-regulate multiple ribosomal protein genes and exhibit compromised protein translation, suggesting that Hos2 may regulate noise by modulating translation machinery. Consistently, treating cells with translation inhibitors as well as mutations in several Hos2 targets, *RPS9A*, *RPS28B*, and *RPL42A*, led to increased noise. Our study provides an effective strategy for identifying noise regulators and sheds light on how cells regulate non-genetic variation through protein translation.

868F **Unraveling the complex world of drug resistance tradeoffs** Kara Schmidlin[1], Sam Apodaca[1], Alexander Sastokas[1], Daphne Newell[1], Grant Kinsler[2], Kerry Geiler-Samerotte[1] [1]CME, Arizona State University, [2]Department of Bioengineering, University of Pennsylvania

There is growing interest in designing multidrug therapies that leverage tradeoffs to combat resistance. Tradeoffs are common in evolution and occur when, for example, resistance to one drug results in sensitivity to another. Major questions remain about the extent to which tradeoffs are reliable–specifically, whether the mutants that provide resistance to a given drug all suffer similar tradeoffs to other drugs. This question is difficult because the drug-resistant mutants observed in the clinic, and even those evolved in controlled laboratory settings, are often biased towards those that provide large fitness benefits. Thus, the mutations (and mechanisms) that provide drug resistance may be more diverse than current data suggest. Here, we perform evolution experiments utilizing lineage-tracking to capture a fuller spectrum of mutations that give yeast cells a fitness advantage in fluconazole, a common antifungal drug. We then quantify fitness tradeoffs for each of 774 evolved mutants across 12 environments, finding that these mutants group into 6 classes with characteristically different tradeoffs. Their unique tradeoffs may imply that each group of mutants affects fitness through different underlying mechanisms. Some of the groupings we find are surprising. For example, we find some mutants that resist multiple drugs in isolation do not resist their combination, and some mutations to the same gene have different tradeoffs than others. These findings, on one hand, demonstrate the difficulty in relying on consistent or intuitive tradeoffs when designing multidrug treatments. On the other hand, by demonstrating that hundreds of adaptive mutations can be reduced to a few groups with characteristic tradeoffs, our findings empower multidrug strategies that leverage these tradeoffs to combat resistance. Finally, by grouping mutants that likely affect fitness through similar underlying mechanisms, our work guides efforts to map the phenotypic effects of mutation.

869F **From macro- to microevolution in the yeast *S. cerevisiae*** Walter P. Pfliegler[1], Alexandra Imre[2], Hanna V. Rácz[3], Bálint P. Németh[3], Andrea P. Harmath[3], Katalin P. Pappné Murvai[3], Rizagul P. Bazenova[3], Malika P. Kultazina[3], Marium P. Farooq[3], Reda M. Lemsali[3], Zsuzsa P. Antunovics[4], Renátó P. Kovács[5], István P. Pócsi[3] [1]Molecular Biotechnology and Microbiology, University of Debrecen, [2]Department of Chemical and Biomolecular Engineering, North Carolina State University, [3]Department of Molecular Biotechnology and Microbiology, University of Debrecen, [4]Department of Genetics and Applied Microbiology, University of Debrecen, [5]Department of Medical Microbiology, University of Debrecen

The yeast *Saccharomyces cerevisiae* is ubiquitous in the food industry and widely used as a probiotic. It may become a long-term commensal and even an opportunistic pathogen. In terms of macroevolution, many of the species' described clades are specifically adapted to traditional or modern industrial fermentation environments. Extensive microevolutionary phenomena, as genome structure variations, and the ability for rapid adaptation are also very characteristic for these yeasts. We aimed to study the phenomena of macro- and microevolution in the genomes of human isolates of the species, by expanding the number of sequenced genomes from clinical samples and food sources (sourdough, lambic, mead, animal feed, etc.) and by conducting in vitro and in vivo experimental evolution.

We sequenced 200 isolates from food and clinical samples, compared whole genome sequences of these along with 1400 previously sequenced samples of food/industrial and human origin together with metagenome-derived yeast genomes, and analyzed *in vitro* and *in vivo* experimentally evolved lineages of three yeast strains to understand how domestication and deliberate breeding affected the adaptability and pathogenic potential of *Saccharomyces* throughout its shared history with humans.

Our results on the phylogenomic relationships of isolate genomes and metagenome-derived yeasts highlighted several industrial clades that are able to colonize the human host at least temporarily. We could also recreate genome structure variations among clinical and food isolates using experimental evolution. We show that genomic characteristics and breeding history had remarkable effects on the in-host microevolution especially in the case of polyploid baking strains. These preferably colonize the female genital tract and display large-scale and rapidly arising genome structure variations independent of meiotic processes. Such an anatomic niche preference and such levels of variation are not characteristic to human isolates of other clades. Similar genome structure variations were characteristic to baker's yeasts evolving in sourdough as well. Remarkably, our phylogenomic analysis identified novel associations between clinical and food industry strains, and also showed that isolates from a two Hungarian hospitals are so diverse that they represent almost all known clades that have ever been recorded from human hosts, although with very different abundances, highlighting the importance of extended sampling efforts.

870F **Predicting population evolution in response to fluctuating environmental conditions** Esdras Tuyishimire, Elizabeth G King Biology, University of Missouri

Undeniably, changes in environmental conditions affect an organism's cellular and physiological functions, which extends to the observed population's behavior regarding genetics. The variability in selection pressure leads to genetic variations in a population that cannot be predicted by the current models. The main goal of the current study is to predict the genotypic and phenotypic changes within a population in response to varying environmental conditions. Advances in computational and genomic technology have allowed scientists to build models to understand the genomic processes underlying the changes we observe in populations under different selection pressures. Yet, results from theoretical models tend not to be consistent with those from empirical models. This study combines quantitative and population genetics theories to make models that can accurately predict population evolution in response to fluctuating environmental conditions. We use a forward evolutionary simulator, SLiM v4.1, to model a quantitative trait while considering a combination of four parameters. We deployed neutral, constant, and fluctuating selection models. Locus effects are derived from a random exponential distribution, with most having a small effect. We used spectral analysis to find the periodic patterns of selection. The results show that monogenic model behaves differently from oligogenic and polygenic models. They also suggest that predicting polygenic and oligogenic models might be easier than the monogenic model. Both allele frequencies and the mean population phenotypes had distinctive patterns for the fluctuating selection models compared to neutral and constant selection models. However, neutral selection tended to behave like complex fluctuating selection, but spectral analysis could differentiate both models. In conclusion, it is possible to predict the effect of fluctuating selection if the assumptions of our models are held, and the models can be extended to predict complex systems like the ones we observe in nature. For future study, we recommend simulating a model that considers additional parameters, for instance, fluctuating population, new mutation, various fitness functions, and other genetic components like epistasis and dominance. This research contributes to understanding the fundamentals that drive population evolution and how the populations respond to ongoing climate change.

871F **Developing a model for predicting the fitness of yeast mutants in antifungal drugs by utilizing Singular Value**

Decomposition
Mohammad Hossein Donyavi[1,2], Kara Schmidlin[1,2], Grant Kinsler[3], Kerry Geiler-Samerotte[1,2] [1]School of Life Sciences, Arizona State University, [2]Center for Mechanisms of Evolution, Biodesign Institute, [3]University of Pennsylvania

Evolving populations are complex and dynamic systems, which makes predicting evolutionary outcomes difficult. Two primary challenges that hinder evolutionary predictions are the extensive scope of genetic target of adaptation and the variable effects these mutations, even those that appear similar, have on phenotype. Antimicrobial resistance continues to pose a serious threat to human health. As the development of new drugs has stalled the ability to predict how mutations behave in a wide range of existing drugs could be invaluable for informing more effective sequential drug treatment strategies. Here, we used a dataset consisting of 774 drug-resistant yeast lineages to train a prediction model via Singular Value Decomposition (SVD). The dataset includes fitness measurements for all 774 yeast lineages in 12 different conditions each of which represents a different concentration or combination of fluconazole (Flu), a common antifungal drug, and radicicol (Rad), an Hsp90 inhibitor. Our study builds upon previous work by investigating how the prediction accuracy changes when we train our model on different subsets of the data. For example, we find we can achieve more accurate fitness predictions in multidrug conditions (Flu + Rad) when training the model on data from Flu environments rather than Rad environments. As this dataset contains a diverse collection of mutations measured in multiple conditions we have the power to address some of the problems that make predicting evolution difficult.Further, this work provides valuable information about when and why mutations that provide drug resistance are more difficult to predict. Finally, the foundational knowledge gained here contributes to the potential incorporation of predicted evolutionary outcomes into designing sequential drug treatments for clinical use.

872F **The Histone Deacetylase Hos2 Regulates Protein Expression Noise by Modulating Protein Translation Machinery** Wei-Han Lin, Florica Jean Ganaden Opoc, Chia-Wei Liao, Jun-Yi Leu Institute of Molecular Biology, Academia Sinica

Non-genetic variations may come from expression noise at the transcript or protein levels, resulting in cell-to-cell heterogeneity within an isogenic population. While cells have developed strategies to reduce expression noise in some cellular functions, this heterogeneity can also facilitate different levels of regulation and provide evolutionary benefits in fluctuating environments. Although several general characteristics of cellular noise have been revealed, detailed molecular pathways underlying noise regulation remained elusive. Here, we established a dual-fluorescent reporter system in *Saccharomyces cerevisiae* and performed experimental evolution to search for mutations increasing the protein expression noise. By analyzing evolved cells using bulk segregant analysis coupled with whole-genome sequencing, we identified the histone deacetylase, Hos2, as a negative protein expression noise regulator. *hos2* mutants down-regulated multiple ribosomal protein genes, leading to compromised protein translation. It suggests that Hos2 may regulate protein expression noise by modulating translation machinery. Consistently, treating cells with translation inhibitors as well as mutations in several Hos2 targets, *RPS9A*, *RPS28B* and *RPL42A*, led to increased protein expression noise. Our study provides an effective strategy for identifying protein expression noise regulators and also sheds light on how cells regulate non-genetic variation through protein translation.

873F **Functional validation of reported genes associated with skin pigmentation in a Native American Caribbean population** Kathryn A Early, Thaddeus D Harbaugh, Victor A Canfield, Keith C Cheng, Khai C Ang Penn State College of Medicine

Skin pigmentation is a phenotype that varies widely between populations of different genetic ancestries. To investigate the genetic determinants of skin color variation between East Asian/ Native American and African ancestries, we sought a Native American population with African ancestry and minimal European contribution that exhibits a wide range of skin pigmentation. The Kalinago, a Native American population from the Commonwealth of Dominica in the Caribbean, fulfill these criteria. Genomic analysis using Admixture of 458 individuals residing in the Kalinago Territory of Dominica revealed genetic contribution of approximately 55% Native American, 32% African, and 12% European ancestry, reflecting the highest reported Native American genetic ancestry among Caribbean populations. Their skin pigmentation measurements ranged from 20 to 80 melanin units, and averaged 46. The most pervasive European skin color alleles, $SLC24A5^{A111T}$ and $SLC45A2^{L374F}$ had allele frequencies of 0.14 and 0.06, and the calculated single allele effect sizes were –6 and –4, respectively. Native American genetic ancestry alone was associated with a pigmentation reduction of 24 to 29 melanin units in the Kalinago. However, the genetic variants responsible for this skin lightening effect remains to be identified, as none of the previously reported polymorphisms associated with skin color in the Native Americans caused a measureable reduction in pigmentation in the Kalinago. In particular, none of the variants proposed to contribute towards skin pigmentation differences between Native Americans and Africans in the genes *CNKSR3 (*rs6917661), *IPCEF1 (*rs2333857), *EFGR* (rs12668421 and rs11238349) and *MFSD12 (Y182H)* reached genome-wide significance, or, in the case of the latter, reached fixation in the Kalinago. To study the potential contributions of these genes towards human skin pigmentation differences and elucidate their role in melanogenesis, we will report on functional validation studies using antisense morpholino (MO) knock-downs of *CNKSR3*, *IPCEF1*, *EGFR*, and *MFSD12* orthologues in zebrafish (*Danio rerio*).

874S **Inherently having more males makes difference in the response of strains to different mutagens in *C. elegans.*** Sayran Saber[1], Rohit Kapila[2], Rahul Verma[2], Janna Fierst[2] [1]Biological Sciences, Florida International University, [2]Florida International University

Cinorhabditis elegans is a hermaphroditic nematode species, with a rare occurrence of males. Males can arise through successful outcrossing between hermaphrodites and males, resulting in an equal ratio of males to hermaphrodites, or through non-disjunction mutations that lead to the formation of spermatids lacking X chromosomes in hermaphrodites. Since the frequency of males in the population is roughly equivalent to the mutation rate, it suggests that males are primarily produced through non-disjunction mutations. The variation in male frequency due to mutational input is specific to different strains.

To investigate this further, we induced inbreeding depression in strains of C. elegans, specifically N2, AB1, and CB4856, which inherently exhibit different male frequencies of 0.1%, 1%, and 5%-20%, respectively. We employed Ethyl methanesulfonate (EMS) and Formaldehyde (FA) treatments for five generations to achieve two objectives: 1) assess changes in male frequency and outcrossing rates, and 2) evaluate the fitness consequences of male frequencies under these mutagenized conditions. Our findings demonstrate that a higher presence of males enhances recovery from mutational stress, and the specific mutational composition influences the extent of male-dependent mutational recovery."

875S **Selection on the length of *C. elegans* giant ankyrin (UNC-44)** Matthew S Rich[1], Erik M Jorgensen[1,2] [1]Biology, University of Utah, [2]Howard Hughes Medical Institute

Many proteins act as linkers in the cell, interacting with multiple partners at specific distances to perform their functions. As such, selection must act on the lengths of these proteins, often through—or in spite of—the expansion and contraction of repeats. We study these selective pressures by examining how they affect the evolution and function of one such long, repetitive protein: giant ankyrin. Ankyrins are conserved, ubiquitous proteins that regulate cell shape by linking membrane proteins to the cytoskeleton. All animals with a nervous system have a giant, neuron-specific isoform of ankyrin that encodes thousands of extra residues, often through the inclusion of long repetitive exons. The sequences of giant ankyrin orthologs are not conserved; instead the giant length and the protein's function are conserved.

While studying the role of *C. elegans* giant ankyrin (*unc-44*) on neuron morphology, we found that only the last 100aa of the over 5000aa neuron-specific, repetitive C-terminus is required for function; deleting the rest of the giant isoform causes only subtle phenotypes. Despite this, the repeat content and length of this exon is highly constrained for nearly 100 million years' evolution among nematodes. Gains and losses of the repeats are observed in natural isolates, but the fixed length is maintained in all species. We are localizing giant ankyrin in *C. elegans* neurons and performing barcoded fitness assays to determine what and how much selection is acting on repeat number, as well as using lab evolution experiments to characterize the genomic mechanism by which the exon expands and contracts. Through these experiments, we hope to be able to define the selective pressures acting on the length of the protein to make it seemingly dispensible, yet nearly immutable.

876S **Functional consequences of the rapid evolution of a putative *de novo* evolved gene required for male fertility in *D. melanogaster*** Alexander S Thurber, Brenna K McCormick, Kerry L McDermott, Prajal H Patel, Geoff Findlay Department of Biology, College of the Holy Cross

De novo evolved genes arise from previously non-coding regions of DNA. Some of these genes can evolve functions that become essential to the organism. We previously identified a putative *de novo* evolved gene, *saturn*, which is required for proper sperm production and sperm motility in *Drosophila melanogaster*. The *saturn* gene has evolved rapidly, under positive selection, across *Drosophila* species. To examine the functional consequences of this rapid evolution, we inserted HA-tagged *saturn* orthologs from different *Drosophila* species into *D. melanogaster* flies lacking an endogenous copy of *saturn* and assessed the ability of each ortholog to restore *D. melanogaster* fertility. We observed that orthologs from species closely related to *D. melanogaster* fully restored fertility, while more divergent orthologs were less effective. The ortholog of *D. ananassae*, in particular, yielded fertility results similar to that of saturn null males. Corresponding to the lack of fertility rescue, the *D. ananassae* ortholog showed a different subcellular localization pattern in developing spermatids. While native Saturn protein and closely related orthologs localized to the nuclear periphery, the *D. ananassae* ortholog localized to elongating sperm tails, suggesting a potential mismatch between this ortholog and other proteins in *D. melanogaster* with which Saturn may normally interact. We are currently raising an antibody to the *D. ananassae* Saturn ortholog to investigate its localization in this species. We are also testing the importance of predicted nuclear localization signals in the *D. melanogaster* ortholog for the protein's function in this species. These experiments show how testing the consequences of evolutionary divergence can lead to hypotheses about the functions of novel proteins. As a second example of this approach, we have also initiated experiments to test the functionality in *D. melanogaster* of orthologs of another, more slowly evolving *de novo* gene required for male fertility, *goddard*.

877S **Genomic diversity illuminates the environmental adaptation of *Drosophila suzukii*** Siyuan Feng[1], Samuel Degrey[2], Christelle Guedot[2], Sean Schoville[2], John Pool[1] [1]Department of Genetics, University of Wisconsin Madison, [2]Department of Entomology, University of Wisconsin Madison

Biological invasions carry substantial practical and scientific importance, and represent natural evolutionary experiments on contemporary timescales. Here, we investigated genomic diversity and environmental adaptation of the crop pest *Drosophila suzukii* using whole-genome sequencing data and environmental metadata for 29 population samples from its native and invasive range. Through a multifaceted analysis of this population genomic data, we increase our understanding of the *D. suzukii* genome, its diversity and its evolution, and we identify an appropriate genotype-environment association pipeline for our data set. Using this approach, we detect genetic signals of local adaptation associated with nine distinct environmental factors related to altitude, wind speed, precipitation, temperature, and human land use. We uncover unique functional signatures for each environmental variable, such as a prevalence of cuticular genes associated with annual precipitation. We also infer biological commonalities in the adaptation to diverse selective pressures, particularly in terms of the apparent contribution of nervous system evolution to enriched processes (ranging from neuron development to circadian behavior) and to top genes associated with all nine environmental variables. Our findings therefore depict a finer-scale adaptive landscape underlying the rapid invasion success of this agronomically important species.

878S **Investigating post-transcriptional regulatory mechanisms of putative *de novo* evolved genes required for *Drosophila* male fertility** Ishanpepe A Jagusah, Emily J Gualdino, Prajal H Patel, Geoff Findlay Department of Biology, College of the Holy Cross

De novo evolved genes arise when previously non-coding DNA sequences evolve an open-reading frame and a functional promoter. Across animals, these genes are often expressed in the testes, suggesting they could evolve essential roles in male fertility. In testis cells, the expression of many genes is also regulated post-transcriptionally. Thus, the evolution of post-transcriptional regulatory mechanisms may be another important aspect of *de novo* gene birth. We are characterizing post-transcriptional regulatory mechanisms for two putative *de novo* genes that are essential for male fertility: *atlas* and *saturn*. Single-cell RNA-sequencing data show that transcripts from both genes initially appear in pre-meiotic cells, but the encoded proteins are detectable only in post-meiotic spermatids. We found that rescue constructs for each gene that contain both upstream and downstream non-coding sequences restore fertility in null males. However, a *saturn* rescue construct lacking the sequences found downstream of its stop codon does not restore fertility, implying key regulatory regions in the gene's 3' UTR and/or after its transcription termination site. We are currently testing whether inclusion of either of these regions in the rescue construct is sufficient to restore fertility. The *atlas* gene has two isoforms, *atlas*-RA and *atlas*-RB, that differ in the length of their 3' UTRs. The shorter RA isoform is more prevalent in whole-male RNA-seq data and our RT-PCR data. To test whether the RB-specific portion of the gene is required for its function, we used CRISPR to specifically delete this region. Males homozygous for the RB deletion allele were more fertile than atlas null males, but exhibited reduced fertility in sperm exhaustive mating conditions, suggesting that the RB-specific region of the *atlas* gene may be important for maintaining high levels of sperm production. One possible mechanism through which the RB region could affect Atlas protein expression is by serving as a binding site for proteins that affect the localization, stability, or accessibility to ribosomes of *atlas* transcripts. We are performing *in situ* hybridization to test if the localization of *atlas*-RA and *atlas*-RB transcripts differ within the testes. Collectively, these results highlight additional evolutionary steps that may be important for newly evolved genes to acquire essential functions.

879S **It's all in good taste: Premating Isolation in the *Drosophila virilis* subgroup** Amisha Agarwala, Yasir H. Ahmed-Braimah Center for Reproductive Evolution, Department of Biology, Syracuse University

Mate discrimination by either sex can be an important form of premating isolation, but studies have largely focused only on female mate choice. Identifying the traits involved in behavioural isolation poses a significant challenge due to the complexity and environmental sensitivity of multimodal courtship signalling systems. The *Drosophila virilis* subgroup is a promising model to examine the genetic and molecular bases of male mate choice. *D. virilis* males mate with heterospecific (*D. novamexicana*) females at far lower frequencies (~10%) than with conspecific females. This preference is mediated by the cuticular hydrocarbon (CHC) profile of the female, and the genetic basis of the female component of isolation has been mapped to a single Quantitative Trait Locus on the X chromosome. Here, we investigate the genetic and mechanistic bases of male mate preference in *D. virilis* males. We find that the male mate preference is determined by the X chromosome by assaying mate preferences in male F1 hybrids from both reciprocal crosses between *D. virilis* and *D. novamexicana*. To analyse the mechanistic basis of the male mate preference, we examined courtship dynamics in conspecific and heterospecific interactions. *D. virilis* males tap the female abdomen with their foreleg tarsi during courtship, and this is hypothesised to be the mechanism by which they sample female CHCs. We amputated the foreleg tarsi of *D. virilis* males and assayed their mate preference. We find that mate preference is mediated by gustatory receptors on the foreleg tarsi as tarsi-less males lack a

preference. Localising the genetic basis to the X chromosome and identifying the sensory mechanism for CHC mediated mate preference allows us to leverage targeted functional genomics assays to further identify the genes and receptors involved.

880S **Discovery of adaptive structural genetic variation in repetitive genome using a pangenome graph of *Drosophila melanogaster*** Alejandra Samano, Mahul Chakraborty Department of Biology, Texas A&M University

Understanding the genetic basis of adaptation is a fundamental puzzle in evolutionary biology. Structural genetic variation involving repetitive sequences that comprise copy number variants (CNVs) and transposable elements (TEs) is an important source of adaptive genetic variation. However, a large proportion of CNVs and TEs are invisible to the standard methods used for identifying adaptive variants from population genomics data based on high-throughput short reads. Additionally, the standard approaches relying on mapping short reads to a reference genome suffer from reference biases and can mislead inference of adaptive evolution based on allele frequencies. To solve these problems, we constructed a *D. melanogaster* pangenome graph using highly contiguous genome assemblies of 15 strains from derived, cosmopolitan populations and 10 strains from an ancestral range African population constructed with long reads. We used this pangenome graph to calculate the allele frequency of comprehensive structural variants (SVs) comprising repetitive sequences in 84 lines from five populations of *D. melanogaster*. These populations are collected from diverse geographic regions, from the ancestral range of the species in tropical Sub-Saharan Africa to temperate, cosmopolitan regions in North America, Asia, Europe, and Australia. We identified many previously hidden candidate adaptive SVs involving TEs and CNVs in these populations. While some candidate adaptive SVs are shared between populations, others are population-specific. Several CNVs and TEs are strong candidates for explaining adaptive phenotypic variation in body and wing size, toxin resistance, thermal stress tolerance, lifespan, diapause incidence, and lifetime fecundity. Using reporter gene assays and CRISPR-Cas9 mediated allele replacements, we investigated the functional effects of a candidate adaptive duplicate and TEs associated with ethanol metabolism and Insulin signaling, respectively, to understand their adaptive significance. Our data suggest that the duplicate CNV contributes to adaptive high ethanol tolerance in temperate populations, and the TEs contribute to adaptive variation in life history traits as an allelic series. Our results demonstrate the utility of pangenome graphs in discovering novel adaptive SVs and highlight the role both large and small shifts in allele frequency of SVs play in adaptive trait variation in *D. melanogaster*.

881S **Genomics of postponed reproduction in *Drosophila melanogaster*** Giovanni A Crestani[1], Karen Walsh[2], Alejandro Moran[2], Hannah S. Dugo[1], Parvin Shahrestani[2], Molly K Burke[1] [1]Integrative Biology, Oregon State University, [2]California State University, Fullerton

Evolve and resequence (E&R) experiments with model organisms have the potential to reveal the genetics underlying complex traits and investigate the molecular dynamics of adaptation. Here, we sequence the genomes of Drosophila melanogaster populations experimentally-evolved for postponed reproduction for over 30 generations. These populations, now maintained on a 70-day generation cycle, rapidly evolved a suite of life-history phenotypes that differ from control populations kept on a standard 14-day generation cycle. These populations live significantly longer than controls and exhibit many correlated phenotypes including increased measures of stress resistance such as improved immune defense following infection with an entomopathogenic fungus. The experimental system we used involves four selection treatments, each consisting of five replicate populations. The two selection treatments on a 70-day (O-type) cycle differ in their recent evolutionary history; one treatment (OB) was derived from populations that had always been reared on a 14-day generation cycle, and the other (OBO) was derived from populations that had been recently reverted from a 70-day to 14-day generation cycle. From those same ancestral populations, we derived control treatments that maintain a 14-day (B-type) cycle; one (nB) that has always been on a 14-day cycle and another (nBO) derived from the same populations that founded the OBO treatment. Each of those four selection treatments represents a unique combination of past and present life-history phenotypes. By comparing the genomes of populations of the O-type selection regimes to those of the B-type selection regimes, we can test hypotheses about the genetic basis of longevity and correlated phenotypes in Drosophila. By comparing the genomes of populations of the same selection treatment, but with different evolutionary histories, we can test hypotheses about the degree to which past evolution constrains future adaptation. Using pooled-population sequencing, we collected whole-genome allele frequency data from all populations at multiple time points over 30 generations of O-type selection. Preliminary results suggest that allele frequencies are strongly predicted by selection treatment, but not by recent evolutionary history, consistent with prior work. We conclude that for standing genetic variation, adaptive differentiation of allele frequencies occurs rapidly, as does evolutionary convergence within selection regimes.

882S **Whole fly 16s microbiome profiles following long-term dietary selection of an outbred genetically diverse population of *Drosophila melanogaster*** Peyton Warren, Garrison Miller, Gaurav Kandlikar, Enoch Ng›oma Division of Biological Sciences, University of Missouri

Aging is a deleterious life history trait that constrains fitness and healthy longevity and has evolved in most species in

response to relaxation in selection late in life. Most efforts to pin down the causes and correlations of nutrition-based aging have focused on the analysis of genomic sequences and transcriptomic profiles, but there remains a large variation in aging phenotypes that is unaccounted for. In this study, we explore the effects of long-term nutritional selection on age-specific microbiome variations. We used large populations of *Drosophila melanogaster* originally maintained for 65 generations under three nutritional regimes: fluctuating protein content, deteriorating protein content, and constant high sugar. After at least 20 generations of inbreeding in common garden, we investigated the age-specific effect of long-term dietary selection on microbiome composition in *D. melanogaster*. We randomly sampled from six inbred selection lines in each selection regime and two lines from an experimentally unevolved population to serve as a control. From each replicate, we assayed whole female flies for 16S rRNA sequencing at three timepoints: 15, 35, and 60 days post-eclosion. Through independent species analysis, we identified 43 bacterial genera that signify significant changes across adult lifespan and 8 genera significantly different between dietary selection regimes. Further analysis of microbial phyla and genera composition suggest putative roles in immunological aging.

883S **A screen for modifiers in the sex determination pathway of *Drosophila melanogaster*** Frederick M Xu, Andrew G Clark, Daniel A Barbash Molecular Biology and Genetics, Cornell University

Sex determination mechanisms vary extensively across taxa, but how much variation exists *within* a sex determination pathway is not fully known. In *Drosophila*, sex is determined by counting five X-linked Signal Elements (XSEs) that activate the primary sex determination gene *Sex lethal* (*Sxl*). In females, there is sufficient XSE dosage from the two X chromosomes to activate *Sxl*; however, in males, the single X chromosome provides insufficient XSE dosage, and *Sxl* remains inactive. Sxl proteins go on to regulate somatic sex differentiation and dosage compensation pathways.

Thomas Cline previously found extensive variation across wildtype *Drosophila melanogaster* strains for sensitivity to perturbations in sex determination. The progeny sex ratios of some strains skewed heavily female-biased when XSEs were duplicated, and others skewed heavily male-biased when *trans*-heterozygous for XSE and *Sxl* LOF mutations—but strains more sensitive to one sex-specific lethality were more resilient to the other. Furthermore, these skews varied in intensity, suggesting an anticorrelated continuum of sensitivity to sex determination perturbations. This begs the question: what is causing this variation?

To genetically map the factors involved, we utilized the Drosophila Synthetic Population Resource (DSPR), a collection of ~1700 Recombinant Inbred Lines (RILs) generated from 15 unique Founder lines. We crossed males from mutant *D. melanogaster* lines containing XSE + *Sxl* LOF mutations or XSE duplications to virgin females from each RIL and scored the progeny sex ratios. We used these data to make QTL maps (at a resolution of ~0.5 – 2 cM) of genomic regions associated with significant signals of sex-specific lethality. We further pared our list of candidate genes within these QTL peaks by scoring the progeny sex ratios of crosses between these same mutant *D. melanogaster* strains with deficiency lines, reasoning that deficiencies should recapitulate the same sex ratio distortion phenotype as any LOF variants segregating in the DSPR. Additionally, several QTL peaks contain known sex determination or dosage compensation genes (*Sxl*, the *upd* gene family, *msl-3*, and *tra*), while other QTL peaks potentially implicate novel genes in either pathway. Using SNP data for each of the Founder lines, we are also identifying potential polymorphisms underlying the sex-specific lethality phenotype for each of the candidate genes.

884S **Gene regulatory targets of selection for enhanced heat tolerance in *Drosophila melanogaster* embryos** Kylie M. Finnegan, Brent Lockwood Biology, University of Vermont

Sensitive life stages may be a selective sieve through which selection acts to establish the genetic makeup of a population. Previous work has shown divergence in heat tolerance at the embryonic stage among populations of *Drosophila melanogaster.* Yet, we lack an understanding of the molecular mechanisms that underlie genetic variation in heat tolerance in this system. Given that thermal tolerance is a complex physiological trait, and that previous work has demonstrated the critical role of gene regulation for heat tolerance, we hypothesized that gene regulatory variation is the primary basis of thermally adaptive variation in heat tolerance. We utilized a combinatorial approach of lab selection and introgression, along with whole genome sequencing and RNA sequencing, to identify the gene regulatory basis of thermal adaptation in *Drosophila melanogaster* embryos. We identified individual genes and gene networks whose expression patterns were targets of thermal selection. We found that some of these genes colocalize to highly variable polymorphic regions across the genome. We discuss the potential functional roles of these genes in the physiology of temperature adaptation. Our results help to elucidate the mechanisms by which thermal tolerance is maintained and inherited in locally adapted populations.

885S **GxGxE for performance traits across 90 mitonuclear genotypes under mitochondrial stress in Drosophila** David M Rand[1], Leah Darwin[2], Yevgeniy Raynes[1], Jared Ingram[1], Faye Lemieux[3] [1]Department of Ecology, Evolutionary and Organismal Biology, Brown University, [2]Computational Biology & Ecology, Evolution and Organismal Biology, Brown University, [3]Department

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Mitochondria don't just make ATP, they are hosts to multiple catabolic and anabolic pathways that maintain cellular metabolism and sense internal and external environmental stressors. Proper cellular and organismal function requires coordinated expression of the 37 mitochondrial-encoded and >1200 nuclear-encoded genes that have been interacting for more than 1 billon years. Disruption of these complex interactions can cause a variety of diseases and influence adaptive evolution in heterogeneous environments. To advance our understanding of how these complex genetic interactions influence organismal fitness we have constructed a panel of 90 mitonuclear genotypes built from 22 different mtDNAs (10 Zimbabwe, 10 Bejing, from D. melanogaster plus D. simulans and D. yakuba mtDNAs) placed onto two nuclear chromosomal backgrounds (OreR and DGRP375) in duplicate. Each genotype was cultured on standard Drosophila diet and a diet containing 25 mM rotenone, a natural pesticide that inhibits the activity of mitochondrial complex I, NADH dehydrogenase. Climbing speed, flight ability, development time and body weight were measured in adult males and females from each diet. This design allows for partitioning of mtDNA effects, nuclear genotype effects, diet environment, and their interactions in models that test for GxGxE effects. There were strong mitonuclear epistatic effects across the phenotypes, with further modification of epistasis by the rotenone treatment. The mtDNA, nuclear and environmental effects were different for each phenotype. OreR nuclear genomes were more sensitive to rotenone than DGRP375, but mtDNA haplotypes modified this effect. The outgroup mtDNAs of D. simulans and D. yakuba had climbing and flight performances comparable to D. melanogaster mtDNAs despite 100s of substitutions in these mtDNA genomes. Unexpectedly, certain outgroup mtDNAs rescued the detrimental effects of rotenone on climbing. Overall, the analyses identify specific mtDNA and nuclear genome pairs that show sensitivity or resistance to rotenone, amenable to further genetic mapping of epistatic (GxG) and environmental (GxE) interactions.

886S **Mechanisms of adaptation to oral infection in *D. melanogaster* upon experimental evolution** Tânia F Paulo[1], Priscilla A Akyaw[2], Tiago Paixão[2], Élio Sucena[2,3] [1]Infectious Diseases, Boston Children's Hospital. Harvard Medical School, [2]Instituto Gulbenkian de Ciencia, [3]Department of Animal Biology, Faculty of Sciences, University of Lisbon

In natural environments, metazoan hosts are constantly enduring the action of pathogens. These interactions constitute strong evolutionary motors that have driven the emergence of the immune responses.

As an animal model of reference, *Drosophila*'s immune response has been well characterized. However, most studies have relied on inbred lines, kept under laboratory conditions, with relatively weak ecological and evolutionary relevance. However, recent work has begun to study *Drosophila* immunity encompassing using natural pathogens in populations with abundant genetic variation, including previous work in the lab that established experimentally evolved populations of *D. melanogaster* under several pathogen challenges. In one such regime, an outbred population of *D. melanogaster* was selected against oral infection (BactOral) with a natural pathogen, *Pseudomonas entomophila*, acquiring after 6 generations of selection, a significantly increased survival upon infection, compared to the Control regime.

To uncover the mechanistic bases of the rapid evolutionary trajectory of BactOral we performed a multi-level phenomenological characterization, alongside transcriptomic profiling. We tested several phenotypes relevant against oral bacterial infections, including feeding and bacterial defecation rates, bacterial load dynamics, and AMP expression levels. We concluded that adaptation in BactOral relied on a combination of higher resistance and possibly stronger disease tolerance.

We conducted RNA-Seq on whole-bodies and on guts at 4 timepoints, revealing differences between populations during both infection progression and resolution. These results agreed with the previous observations of increased resistance of BactOral, at the gut level. Additionally, at the whole-organism level, BactOral exhibits an earlier downregulation of immune effectors that correlates to the its higher disease tolerance.

Finally, using conditional RNAi, we functionally validated the role of the candidate genes *Drosomycin-like 2* and *Lactate dehydrogenase* in the immune response of Drosophila, possibly in the control of bacterial loads and localized gut immunity.

In sum, this work systematically addresses several layers of the immune response, gaining a deeper understanding of how immunity be shaped by evolution. Additionally, it highlights that resistance and disease tolerance can evolve in parallel, synergizing or additively contributing to higher fitness.

887S **Rapid evolution of piRNA clusters in *Drosophila melanogaster* ovary** Satyam P Srivastav, Cedric Feschotte, Andrew G Clark Molecular Biology and Genetics, Cornell University

The piRNA pathway is a conserved mechanism to repress transposable element (TE) activity in the animal germline via a specialized class Argonuate proteins called Piwi and small RNAs called piwi-interacting RNAs (piRNAs). piRNAs are produced from discrete genomic regions called piRNA clusters (piCs). While the molecular processes by which piCs function are relatively

well understood in *Drosophila melanogaster*, much less is known about the origin and evolution of piCs in this or any other species. To investigate piC origin and evolution, we use a population genomics approach to compare piC activity and sequence composition across 15 geographically distant strains of *D. melanogaster* with high quality long-read genome assemblies. We perform annotations of ovary piCs and genome-wide TE content in each strain. Our analysis uncovers extensive variation in piC activity across strains and signatures of rapid birth and death of piCs. Most TEs inferred to be recently active show an enrichment of insertions into old and large piCs, consistent with the previously proposed 'trap' model of piC evolution. By contrast, only a small subset of active LTR families is enriched for the formation of new piCs, suggesting that these TEs have higher proclivity to form piCs. Thus, our findings uncover processes leading to the origin of piCs and principles of piC and TE co-evolution. We propose piC evolution begins with the emergence of piRNAs from a few specific LTR retrotransposon insertions that subsequently expand by accretion of other TE insertions during evolution to form larger 'trap' clusters. Our study shows that TEs themselves are the major force driving the rapid evolution of piCs.

888S **Flight, Form, and Fitness: Unveiling the Robustness of Adaptation** Saudat Alishayeva, Luisa F Pallares Friedrich Miescher Laboratory, Max Planck Society

Biological systems have a remarkable dual capacity: they are robust to mutations and developmental noise, while also evolving and responding dynamically to their environment. To gain a better understanding of robustness and evolvability, we conducted a comparative analysis of wild-type *Drosophila* reared under control conditions and those exposed to a sucrose and glucose-rich diet. Our focus encompassed three key components of phenotypic robustness: population-level analysis of wing shape morphology, developmental stability reflected in wing asymmetry, and flying performance.

Surprisingly, we observed a reduction in behavioral variation in populations reared on high-sugar diets that exhibited robust flight performance. Our results indicate that individuals exposed to stressful dietary conditions also demonstrate rapid and substantial changes in morphology and fluctuating asymmetry. Despite these alterations, functional capacity for flight was maintained, highlighting a remarkable resilience in the face of underlying morphological shifts. Tracking these phenotypic traits over 15 generations allowed us to discern both immediate and delayed adaptive responses to environmental stress.

To further probe the environmental role in adaptability, we initiated experiments implementing stabilizing selection on flies raised under two distinct conditions. In each generation, we artificially reduced variation in flight performance by selecting flies close to the mean. Remarkably, flies reared on control food exhibited faster adaptation to truncating selection, manifesting in reduced flight variation within just five generations. In contrast, flies subjected to food enriched with sugar displayed a noisier and less predictable response, suggesting a diminished adaptability to artificial selection. The fluctuations in variation observed are likely a result of genotype-by-environment interactions (GxE), influencing the heritability pattern for flight performance.

In conclusion, our study sheds light on the dynamic nature of phenotypic responses to environmental stress and the subsequent implications for adaptive evolution. The ability of organisms to maintain functional traits amidst morphological and symmetrical alterations underscores the complexity of the relationship between genotype and phenotype. These findings detangle the speed of adaptive responses in terms of behavior, morphology, and developmental stability, with potential applications in understanding and managing evolutionary processes in response to stress.

889S **Isofemale lines, genomic environment and evolution** Guy F Barbato Biology, Stockton University

Two years ago, we began collecting wild *Drosophila melanogaster* from the homes of our undergraduate Genetics lab students. Training students in the care and breeding of *Dmel*, our intent is to quantify naturally occurring variation using both morphometric, physiological and behavioral measurements. Further, we began having undergraduate research students extract gDNA from the lines to begin Oxford Nanopore sequencing.

The isofemale lines were created from student collections by using a single female fly as the progenitor of the line. In each case, three three replicate populations were started using different females. The populations were then inbred using full-sib matings for at least ten generations prior to collecting phenotypic and genomic data. We have 128 individual isofemale lines derived from 50 collection sites.

During routine reproduction of the lines, pupae are counted at 10d post-mating, with the lines having 74 ± 44.7 pupae (CV=61%). Having such large differences in reproductive fitness, we assessed gamete production in both male and female gonads of mature flies. Ovariole counts ranged from 8 – 35 per ovary per female, while spermatid counts between low and high fitness lines was an order of magnitude. The relationship between gamete production and fitness was multidimensional and not due to any single factor – meaning the poor fitness could be due to either male or female gamete production, or gametic incompatibilities. Given the nature of the lines, we were not entirely surprised to see multifaceted relationships arise.

We have also observed a three-fold difference in body size among the lines and there are large differences in sexual dimorphism with many lines exhibiting no sexual dimorphism of body size. Conversely, 10% of the lines exhibit the opposite sexual dimorphism of size than expected. That is, males are consistently larger than females.

A major thrust of the project is to obtain genomic data from the lines using the Oxford Nanopore technologies. Given that all our research is being executed by undergraduate students, we have had to develop biochemical and genetic workflows that can accommodate their class schedules. Focusing on a team-based approach, be now have specific workflows to extract poly-A-mRNA from gonads and gDNA from both live and frozen flies. We have been successful in sequencing both molecules using the MinION MK1C (v.9.4.1 chemistry) and are in the process of upgrading to the new flow cells. Existing data are being analyzed in my senior-level BioInformatics class.

890S **Mixed Wolbachia infections resolve rapidly during in vitro evolution** Cade Mirchandani[1], Pingting Wang[1], Maximilian Genetti[1], Jodie Jacobs[1], Evan Pepper-Tunick[2], Russell Corbett-Detig[1], Shelbi L Russell[1] [1]Biomolecular Engineering, University of California Santa Cruz, [2]Molecular Engineering Sciences Institute, University of Washington

The intracellular symbiont *Wolbachia pipientis* evolved after the divergence of arthropods and nematodes, but it reached high prevalence in many of these taxa through its abilities to infect new hosts and their germlines. Some strains exhibit long-term patterns of co-evolution with their hosts, while other strains are capable of switching hosts, making them important factors in symbiont-based biological control. However, little is known about the ecological interactions that occur when a promiscuous strain colonizes an infected host. Here, we study what occurs when two strains come into contact in host cells following horizontal transmission and infection. We focus on the faithful wMel strain from *Drosophila melanogaster* and the promiscuous wRi strain from *Drosophila simulans* using an in vitro cell culture system with multiple host cell types and combinatorial infection states. Mixing D. melanogaster cell lines stably infected with wMel and wRi revealed that wMel outcompetes wRi quickly and reproducibly. Furthermore, wMel was able to competitively exclude wRi even from minuscule starting quantities, indicating that this is a deterministic attribute, independent of the starting infection frequency. This competitive advantage was not exclusive to wMel's native *D. melanogaster* cell background, as wMel also outgrew wRi in *D. simulans* cells. Overall, wRi is less adept at in vitro growth and survival than wMel and its in vivo state, revealing differences between cellular and humoral regulation. These attributes may underlie the observed low rate of mixed infections in nature and the relatively rare rate of host-switching in most strains. Our in vitro experimental framework for estimating cellular growth dynamics of *Wolbachia* strains in different host species, tissues, and cell types provides the first strategy for parameterizing endosymbiont and host cell biology at high resolution. This toolset will be crucial to our application of these bacteria as biological control agents in novel hosts and ecosystems.

891S **Experimental evolutionary genomics of herbivore adaptation to toxic host plants** Noah Whiteman[1], Andy Gloss[2], Joy Bergelson[2], Diler Haji[1] [1]Department of Integrative Biology, University of California, Berkeley, [2]Department of Biology, New York University

A major problem in evolutionary biology is why and how herbivores evolve different host ranges. Oligophagy can arise through phenotypic plasticity or when there is additive genetic variation for alternative alleles. In 1953, Howard Levene proposed that spatially- or temporally varying selection can maintain functional genetic variation, enabling species to exploit a diversity of niches. Insect herbivores are biologically well suited to test this model because adults mate randomly and offspring are exposed to – and are often restricted – to different host plant chemical environments over development. We tested the Levene model by experimentally evolving the herbivorous leaf-mining drosophilid fly *Scaptomyza flava* for 10 generations on monocultures or mixtures of two natural and chemically divergent mustard host plants, *Barbarea vulgaris* and *Turritis glabra*, and then compared our experimental findings with wild-caught populations from both host plants. Scaptomyza flava evolved increased fitness and a home-court advantage on *Barbarea* and *Turritus* monocultures. These fly populations showed rapid genome-wide differentiation within 6-8 generations. Genomic differentiation occurred in opposite directions between the monocultures while mixture treatments showed intermediate differentiation. Regions of the genome exhibiting heightened differentiation between monocultures contained genes predicted to play roles in cell maintenance, xenobiotic metabolism, and stress response.

892S **Exploring the Genetic Basis of the Tan Streak Mutation in Deer Mice** Isabel Carino-Bazan[1], Shuonan He[2], Hopi Hoekstra[2] [1]Biological Sciences, Lehigh University, [2]Harvard University

Coloration varies vastly amongst organisms, even between individuals of the same species. One such example is *Peromyscus*, which are found across North America and display profound coat color changes as they adapt to distinct environments. A naturally-occurring color morph in *Peromyscus* is known as tan streak (*tns*), which gives mice a distinctive white coat with a tan-dorsal streak. However, the molecular nature of the *tns* mutation is yet to be fully explored. Melanocortin receptor (*Mc1r*)

is an important regulator of mammalian pigmentation synthesis, and deer mice carrying CRISPR-induced *Mc1r* mutations display a strikingly similar coat coloration to *tns* mice. Through our experiments, we aim to test our hypothesis that the *tns* phenotype is due to loss of function of the *Mc1r* gene. To test our hypothesis, the two methods we used were spectral analysis of the flat skin coat color, which allowed us to quantify and compare brightness, hue, and saturation along the dorsal-ventral axis, and genotyping of the *Mc1r* locus. Spectral analysis showed us that brightness, hue, and saturation in *tns* and *Mc1r*-mutants are comparable and that these measurements are significantly higher than those of the wildtype flat skins. From genotyping, we detected four nucleotide mutations, three of which resulted in amino acid changes, in the *Mc1r* coding region of *tns* samples. These two results allow us to deduce that the phenotypic similarity between *Mc1r*-mutants and *tns* mice denotes a genotypic correlation.

893S **Genetic and non-genetic effects on transcriptional and post-transcriptional gene regulation** Mallory A. Ballinger[1,2], David N. Manahan[3], Michael W. Nachman[3] [1]Biology, Utah State University, [2]Ecology and Evolutionary Biology, Cornell University, [3]Integrative Biology, University of California, Berkeley

Determining how both genetic and environmental effects influence the regulation of gene expression is key to understanding the molecular mechanisms of adaptive evolution. However, most studies on gene regulatory evolution have primarily focused on the transcript level (mRNA abundance); we know relatively little about the role of transcript structure (alternative splicing) in adaptive evolution. Due to their recent expansion into new environments, house mice (*Mus musculus domesticus*) provide an excellent opportunity to study genetic and environmental effects on transcriptional and post-transcriptional gene regulation. Here, we use short-read RNA-seq from temperate and tropical wild-derived inbred lines of house mice reared in warm and cold environments to address several key questions regarding gene regulatory evolution: Does variation in alternative splicing and mRNA abundance play complementary and/or contrasting roles in adaptive evolution? What is the relative contribution of *cis*- versus *trans*-regulatory changes to splicing and expression divergence? To what extent are transcriptional and post-transcriptional regulatory mechanisms environmentally dependent? Overall, this work broadens our understanding of the genetic and non-genetic gene regulatory mechanisms underlying adaptive evolution.

894S **Convergent evolution in response to hypoxia in *Peromyscus* mice** Peter Nimlos[1], Jonathan Velotta[1], Nathan Senner[2], Zachary Cheviron[3] [1]University of Denver, [2]University of Massachusetts - Amherst, [3]University of Montana

A powerful approach to understanding the mechanisms of adaptation is to investigate species that have independently evolved to overcome similar environmental challenges. Studying this phenomenon – known as convergent evolution – can help explain the extent to which evolution is repeatable and predictable. However, quantification of convergence has been difficult due to the complexity of intersecting genetic and physiological pathways. High altitude environments provide an apt setting to study convergence as low oxygen availability (hypoxia) and prolonged cold provide intense selection pressures leading to physiological adaptation. The ability to generate heat from aerobic metabolism (thermogenic capacity) is a critical determinant of fitness in wild high-altitude deer mice (*Peromyscus maniculatus*) and is accomplished via contraction of skeletal muscle (shivering). Indeed, populations of deer mice native to high altitude have higher thermogenic capacity relative to their lowland native conspecifics, which is thought to be driven by an increase in the density of oxidative (O2 consuming) muscle fibers. *P. maniculatus* is one of many species in the genus *Peromyscus* that have independently invaded high-altitude environments across North America, making them a unique system to test for convergent patterns of adaptation in thermoregulation. Here, we measured transcriptomic response and whole organism performance of six species of laboratory-raised *Peromyscus* mice native to either low- and high-altitudes and acclimated for six weeks to a gradient of simulated elevation (equivalent to 1,000, 3,000 and 4,500 m a.s.l.). We measured thermogenic capacity during a cold exposure, quantified muscle tissue composition, and sequenced skeletal muscle transcriptomes. Using phylogenetically-informed models, we compared gene expression between species and acclimation treatments and identified shared highland genes putatively differentiated in thermogenesis. We correlated measured thermogenic capacity with expression of candidate genes. The combination of integrated data types, broad phylogenetic sampling, and controlled laboratory acclimations lends substantial comparative power to discern the extent to which within-species acclimation response and across-species evolutionary changes represent adaptations to extreme altitude.

895S **Cross-Species Comparative Transcriptomic Analysis of Embryonic Stem Cells (ESCs)** Yifei Fang, Ellie Duan Animal Science, Cornell University

Embryonic stem cells (ESCs) have huge potential in developmental biology and regenerative medicine due to their unique pluripotent capacity to differentiate into any cell type in the organism. However, the mechanisms underlying this potential are complex and not fully understood. To unravel the complexities of pluripotency, we conducted a comprehensive comparative transcriptomic analysis of ESCs across humans, mice, rats, cattle, pigs, horses, sheep, crab-eating macaques, and marmosets. We aim to identify genes and regulatory networks controlling pluripotency that are evolutionarily conserved and those that

are species-specific. Principal Component Analysis (PCA) revealed greater variations across species than within pluripotency states. The analysis also showed a conserved trajectory among different ESC pluripotency states across species. Notably, human naïve ESCs (nESCs) is more closely resemble mouse primed ESCs (pESCs), indicating fundamental differences in these pluripotent states among different species. The hierarchical clustering analysis shows two major clusters that separate primates and non-primates. Transcriptome-based phylogenetic trees also show mice nESC is closer to humans while macaques pESCs are closer to humans, suggesting the phylogenetic distance between species can be different depending on the pluripotent states of the cells. Consistently, differential gene expression (DEG) analysis among ESCs at different states of pluripotency also showed macaque pESCs are most similar to human pESCs. Additionally, we found that nESCs commonly have more DEGs in interspecies comparisons than pESCs, suggesting the naive state may be more species-specific while the primed state may be more conserved. Moreover, we discovered 14 genes that conserved pESC species in at least three of the species. Notably, the gene C1QL2 is conserved throughout all species studied. Overall, our comprehensive comparative transcriptomic analysis across species revealed that the primed pluripotency state is more evolutionarily conserved than the naive state. These findings contribute to our understanding of stem cell biology and the evolutionary underpinnings of pluripotency.

896S **Ancient human genomic data and linear mixed models identify pervasive polygenic selection in the human genome** Jared E Decker[1], John Miraszek[2], Brooke K. Bowman[3], Caleb J. Grohmann[4] [1]Division of Animal Sciences, University of Missouri, [2]Genetics Area Program, University of Missouri, [3]Biological Sciences, University of Missouri, [4]Institute for Data Science and Informatics, University of Missouri

Identifying the loci responding to selection has been a focus of modern population genetics. Approaches using patterns of DNA variants have successfully discovered hard and soft sweeps in which strong selection changes nucleotide diversity. However, polygenic selection on complex traits results in small allele frequency changes at thousands of loci and does not leave the strong signature of a sweep. Polygenic selection does create an association between allele frequencies and time. We use reverse regression in which a proxy for generation number is fit as the dependent variable in a genome-wide association linear mixed model to identify associations between time and genotype. We analyze 11,386 ancient and modern human samples from the Allen Ancient DNA Resource and their corresponding dates before present. We have previously shown that the genomic relationship matrix in linear mixed model genome-wide association appropriately accounts for the demography of the analyzed population. Here, we also fit principal components from analyses of genotypes and missing genotypes as covariates in the statistical models to produce well-calibrated p-values. We identify 5,184 variants with genome-wide significant associations with date before present. In addition to numerous novel associations, we identify canonical targets of selection in humans, including *SLC45A2* and *SLC24A5*. Associated genes were enriched in gene ontology terms and pathways related to immunity, anatomical structure development, and nervous system development. In even our most conservative models, we find associations with *GRM5* and *NRXN1*, which are known to affect learning phenotypes. These results allow us to understand the complex history of polygenic selection in humans.

897S **Extent and effects of changes in gene dosage on the neo-sex chromosomes of parasitic nematodes** Kevin A Hackbarth[1], Tibeb S Mekonen[1], Julie C Dunning Hotopp[2,3], Eric S Haag[1] [1]Biology, University of Maryland, [2]Microbiology & Immunology, University of Maryland School of Medicine, [3]Institute for Genome Sciences

Brugia malayi and *Onchocerca volvulus* are filarial nematode parasites consequential for public health. In each species, a different autosome fused with the X chromosome to form a neo-X chromosome. As a result, their ancestrally XX/XO systems became XX/XY, where the neo-Y represents a degenerated version of the unattached autosomal homolog. As genes on the neo-Y diverge or are lost, the resulting change in gene dosage creates evolutionary pressures to rebalance expression levels, silence unpaired genes during male meiosis, and adapt the gene content on these sex-linked regions. Here, we characterize neo-X/-Y divergence and reanalyze RNA-seq datasets to reveal dosage compensation and meiotic silencing in *B. malayi* and *O. volvulus*. These findings inform our understanding of sex chromosome divergence, dosage sensitivity, transcriptional regulation, and gametogenesis in these parasitic nematodes and in organisms with neo-sex chromosome evolution generally.

898S **Exploring the distribution of ultra-rare mutations under exponential population growth** Deepjyoti Ghosh[1], Margaret C Steiner[2], John Novembre[2,3], Yuval B Simons[1,2] [1]Section of Genetic Medicine, University of Chicago, [2]Department of Human Genetics, University of Chicago, [3]Department of Ecology & Evolution, University of Chicago

Large databases of whole exome and genome sequencing studies are giving the first glimpse of the spectrum of ultra-rare alleles in human populations. This spectrum is dominated by young alleles which arose during recent explosive population growth, in which human populations increased by a factor of roughly 100,000 in less than 2000 generations. Therefore, understanding the frequency spectrum of ultra-rare alleles (frequency under 10-3) requires models of allele dynamics during such explosive population growth. We use branching processes to show that the distribution of allele frequencies in an exponentially growing population is inversely proportional to the square of the frequency. Additionally, the distribution is proportional to the mutation rate and inversely proportional to the growth rate. We use simulations to show that the scaling

with allele frequency is insensitive to the exact shape of population growth. We corroborate our results using data from whole exome sequencing of a White British population in the UK biobank, showing that the site frequency spectrum is indeed inversely proportional to the square of the frequency. These results suggest that the numbers of ultra-rare mutations in whole genome or exome sequencing studies will grow linearly with study size until the study size has reached about 5 million. At that point, every site in the genome should be segregating, except for evolutionarily constrained sites (with selection coefficient above 1%). Therefore, such large study sizes would produce a full map of all evolutionarily constrained sites in the human genome.

899S **Ancient DNA confirms crossbreeding of domestic South American Camelids in two Pre-Conquest archaeological sites** Alondra M Diaz-Lameiro[1], Jennifer L. Kennedy[2], D. Andrew Merriwether[3] [1]Biology, University of Puerto Rico at Mayaguez, [2]Binghamton University, [3]Anthropology, Binghamton University

The llama (*Lama glama*) and the alpaca (*Vicugna pacos*) are important domesticated species, endemic to South America. South American camelids helped ensure the success of humans in the Andes, much like the horse in Europe. Two wild South American camelids, the guanaco and the vicuña have been proposed as the ancestors of these domestic forms. Some scientists have hypothesized that crossbreeding started after the Spanish conquest in the 1500's, since before this event, indigenous people are thought to have kept both domestic breeding lines separate. In an effort to avoid the confounding effects of crossbreeding, ancient DNA from pre-conquest South American camelids was analysed from sites in Bolivia and Ecuador. Our mitochondrial pre-conquest results for Cerro Narrío in Ecuador show that all ancient samples which do not naturally occur in this region and were likely domesticated camelids, had maternal guanaco ancestry. At the Bolivian site of Iwawi, near Lake Titicaca, matrilineal ancestry from both wild species contributed to the domestic forms. These results help disentangle the complex ancestry of the domestic South American camelids and inform future breeding strategies. Additionally confirm the occurrence of crossbreeding between camelids pre-Spanish conquest.

900S **Genetic and Molecular Bases of Hybrid Dysgenesis in Deer Mice** Wyatt Toure, Riya Rampali, Andrés Bendesky Columbia University

Understanding the mechanisms by which incompatibilities arise between populations and species remains a fundamental problem in evolutionary biology. Three clear patterns of hybrid incompatibility ("rules of speciation") have emerged from over a century of observational and experimental work on hybrids: Haldane's rule (hybrids of the heterogametic sex are more affected), Coyne's rule (the X and Z chromosomes play an outsized role in incompatibilities), and Darwin's corollary (asymmetric hybrid effects). These patterns are phylogenetically broad, yet we still do not have a mechanistic understanding of how they manifest in mammals. Deer mice (genus *Peromyscus)* offer an opportunity to resolve the genetics and molecular biology of hybrid incompatibilities since they exhibit all three rules yet are amenable to quantitative genetic mapping of their incompatibilities. The cross in which *P. maniculatus* is the mother and *P. polionotus* is the father produces viable, fertile offspring but the reciprocal cross produces an embryonic lethal overgrowth that renders this cross inviable, except for rare female survivors. Through QTL mapping, we have revealed that the largest genetic effect contributing to the overgrowth is on the X chromosome and that it only affects males and not females. The QTL in question maps to the X inactivation center, an intriguing result, since male mice are hemizygous for the X chromosome and do not typically undergo X inactivation. Our analysis of RNA-sequencing data has revealed that the QTL associated with overgrowth leads to a global reduction in the level of X-linked transcripts in males, suggesting dosage compensation may be perturbed. Further RNA-sequencing of embryos reveals that dosage compensation perturbations are already present by day 13 of embryonic development and that the expression of imprinted genes is highly perturbed. Ultimately, by providing mechanisms for hybrid incompatibilities between naturally diverging mammalian species, we will reveal insights into the classic rules governing the maintenance and origin of species.

901S **Genomic Evolution of Phi-6 Cystovirus under Heat Shock Treatments** Sara Nayeem, Parnian Pour Bahrami, Sujayashree Jayatirtha Nilogal, Sarosh Syed, Pranav Babu, Sanika Samel, Sonia Singhal San Jose State University

Climate change can lead to increases in temperature over time, and the rate of increase may influence how populations adapt to the changing environment. For example, the number and types of mutations used for adaptation may depend on the rate of environmental change. We address this question in a model system, the RNA bacteriophage phi-6, under high temperatures. Bacteriophages (viruses that infect bacteria) are good candidates to study evolution because they have high mutation rates, high replication rates, and large population sizes, and are harmless to humans. We previously evolved populations of phi-6 for 32 days (approx. 100 viral generations) under high-temperature heat shocks in different treatments. In the Sudden treatment, populations experienced constant heat shocks at 50°C. In the Gradual treatment, heat shock temperatures increased each transfer up to 50°C. Samples were cryogenically stored at each transfer. Based on differences in selective pressures under sudden and gradual environmental change, we expect to see adaptation to heat shocks through a few mutations of large effect

in the Sudden treatment, and adaptation through mutations of smaller effect in the Gradual treatment. Here, we test these hypotheses in our phi-6 lineages. We revive the frozen populations and extract viral RNA from the samples at transfers 8, 16, and 24, representing times one-quarter, one-half, and three-quarters of the full experiment. After reverse transcribing the RNA into cDNA, we sequence the samples to identify and track any changes in the viral genetic material over evolutionary time. Our experiments provide important information on both how viruses specifically adapt to high temperatures and how organisms more broadly adapt under different rates of climate change.

902S **Best practices for identifying fast evolving non-coding elements using PhyloAcc-GT: A cases study using echolocating mammals** Gregg W.C. Thomas[1], Subir B. Shakya[1], Scott V. Edwards[2], Timothy B. Sackton[1] [1]Informatics Group, Harvard University, [2]Organismic and Evolutionary Biology, Harvard University

Comparing the genomes of multiple species in a phylogenetic context has become a common way to study how molecular changes in DNA accumulate over evolutionary timescales. One way to quantify genomic variation is to assess the rate at which it accumulates over time. Doing this, regions of the genome that evolve at varying rates on different lineages can be identified and allow us to infer when and where selection may have played a role in sequence evolution. These inferences at the genotypic level can subsequently be linked to phenotypic evolution. We recently released PhyloAcc-GT, which identifies shifts in nucleotide substitution rate along a phylogeny. Unlike codon-based models, PhyloAcc-GT can be used to infer rate shifts in non-coding elements. PhyloAcc-GT also accounts for phylogenetic discordance between the underlying loci and the species tree. Here, we show how PhyloAcc-GT can be used on a large-scale genome alignment of 241 mammals. We provide workflows and best practices to process a multiple alignment format (MAF) file to identify the putative conserved non-coding elements that PhyloAcc-GT uses as input. We provide Snakemake workflows for conserved element prediction using PHAST. We consider both explicit conserved element prediction and a more flexible identification of genomic regions with a high percentage of significantly conserved sites and with high alignment coverage. We also provide tools to work with large MAF files and extract sub-alignments, which are used as input to PhyloAcc-GT. We demonstrate our workflows by identifying non-coding elements that are convergently accelerated in lineages of echolocating mammals (bats and dolphins).

903S **Inference of selection coefficients while controlling for gene flow using ancient DNA** Xinyi Li[1], John Novembre[2] [1]University of Chicago, [2]Human Genetics, University of Chicago

Studying genetic signatures of natural selection using ancient DNA may help identify functionally important variants. However, the complex demographic history of humans can hide selection signals or lead to spurious discovery. In particular, gene flow from diverged populations can cause rapid shifts in allele frequencies that may be mistaken as selection signatures.

To address this limitation, we developed a new method to infer selection while controlling for the effects of gene flow. We model the allele frequency change of a target population as a function of genetic influx from other source populations, drift, and selection. First, we infer the posterior distribution of migration proportion parameters using SNPs from putatively neutral regions. Second, conditioning on the migration proportions, we estimate the selection coefficients for variants in interest. At each step, to obtain the posterior distribution efficiently, we use variational inference.

To test this method, we applied our method to simulated data. In these simulations, compared to a method that does not account for migration, our approach obtains a more accurate and unbiased selection coefficient. For instance, in the case of 30% pulse migration, an initial frequency of 0.01 in the target population, and a selection coefficient of 0.001, the approach without considering gene flow infers on average a 3-fold larger selection coefficient estimate (s=0.0034 vs. s=0.0012).

We applied our method to publicly available data from 481 individuals from the Carpathian Basin, including samples ranging from 5000 BCE to 900 CE (Mallick et al., 2023). Our method fits two major events of gene flow: 1) from Steppe-like ancestry during the Bronze Age and 2) from Xiongnu-like ancestry in the Medieval period. Correcting for these gene flow events, we recover known selected signals and exclude likely false positives. For instance, our method recovers a selection signal at rs5743810 which was known to be an Fst outlier (posterior mean of s= 0.006 with 95% credible interval [0.00074, 0.012]) while a method that does not account for gene flow does not have enough power (posterior mean of s= 0.004 with 95% credible interval [-0.0019, 0.011]).

While gene flow among populations has been shown to be pervasive across human history, our new method can be a powerful way to infer selection using ancient DNA without biases from gene flow.

904S **Connections between topological data analysis and identity-by-descent in biobank data** Alex Diaz-Papkovich[1], Cole Williams[1], Justin Pelletier[2], Simon Gravel[2], Claude Bherer[2], Sohini Ramachandran[1] [1]Brown University, [2]McGill University

Population structure reflects shared population history. A variety of methods are used to identify structured subgroups in

genetic datasets. One such approach is detecting pairwise shared chromosomal segments that are identical-by-descent (IBD), indicating shared ancestry, followed by clustering of those segments. Having identified IBD clusters, researchers can subsequently perform analyses of demographic history, or study associated biomedical data and environmental variables for these groups.

While IBD clustering is common, it can be computationally taxing. An efficient and now-common dimensionality reduction method in population genetics is uniform manifold approximation and projection (UMAP). UMAP works by preserving the high-dimensional topology of data and is usually applied to genetic variants rather than haplotypes. It can also be paired with density clustering to define clusters without the overhead of chromosomal phasing and parametrization of IBD calling. The UMAP-identified subgroups correlate strongly with demographic history, environmental, and phenotypic data and, similar to results of IBD clustering methods, identify very fine-scaled structure in complex genetic cohorts. Though UMAP clusters are often discussed in studies of genetic cohorts, no explicit connection has been drawn between UMAP clusters and IBD sharing. Such connections can inform researchers of the relationships between processes like coalescence and relatedness, aid in interpretation of non-linear dimensionality reduction, as well as provide an alternative set of computationally cheap and tractable tools to study clusters from structured population in large biobanks.

We present results from CARTaGENE, a cohort of residents of Québec, and the Thousand Genomes Project. We find a strong relationship between the clusters found via UMAP followed by density clustering compared to those found via IBD clustering in CARTaGENE. We use CARTaGENE, the UK Biobank, and the Thousand Genomes data to study how genetic relationships between individuals in data influence visualization and clustering. We discuss the relative strengths of the approaches and potential theoretical connections between topological clustering and IBD clustering.

905S **History repeats itself: Genetic changes underlying convergent phenotypes are revealed by comparative genomics** Nathan Clark[1], Avery Selberg[2], Maria Chikina[3], Sergei Pond[2] [1]Biological Sciences, University of Pittsburgh, [2]Biology, Temple University, [3]Computational and Systems Biology, University of Pittsburgh

A combination of comparative genomics and phylogenetics is increasingly being used to assign genotype to phenotype through an approach called PhyloG2P. Many such methods examine species sharing a convergently evolved phenotype and identify related genes that experienced parallel changes in selection in those species. If adaptive changes in a gene were responsible, positive selection or accelerated rates are expected. If, instead, the phenotype is the degeneration of a trait, relaxed constraint on related genes would be seen as accelerated rates or pseudogenization. Using these expectations, PhyloG2P methods search for positive selection, shifts in evolutionary rate, or pseudogenization associated with the branches over which the convergent phenotype evolved. To date, these approaches have identified new genes and regulatory regions controlling traits important to fitness and health. When applied to blind mammals, PhyloG2P located new ocular genes and enhancer elements. In flightless birds, new enhancers were implicated in wing development. Study of “bald” mammal species identified new microRNAs and enhancers involved in hair follicle formation, and new sperm proteins were found by studying the sperm competition in rodents and primates. A general observation across these studies is that trait losses are more powerful at identifying phenotype-related genes, because once constraint is released it produces a strong rate acceleration that continues indefinitely. Conversely, it is difficult to identify genes underlying phenotypic changes involving adaptive evolution, likely because the responsible positively selected changes were episodic and relatively few. To identify adaptive changes via PhyloG2P, the field requires tools to identify branch-specific bursts of positive selection that are absent from branches not involved in the convergent phenotype. To meet this need, we developed a custom set of likelihood models in BUSTED-PH to identify positive selection that acted specifically on branches with a convergent PHenotype. BUSTED-PH’s application to aquatic mammals identified genes involved in adaptation to diving along with skin and lung traits, presumably due to selection on impermeability and compression. PhyloG2P approaches continue to evolve just as the natural world does in response to its challenges. New developments on the horizon will examine gene expression and genomic indicators of regulatory function with respect to convergent phenotypes.

906S **Expression and Evolution of Alt-ORFs in the Human Genome** Naiqi Zhang[1], April Wei[2], Zachary Ardern[3] [1]Cornell University, [2]Computational Biology, Cornell University, [3]Wellcome Sanger Institute

Many open reading frames (ORFs) with evidence of protein translation are encoded so as to overlap known protein-coding genes, thereby reading a completely different codon sequence from the same nucleotide string.

Despite their functional relevance, the systematic study of protein-coding alt-ORFs in the human genome from an evolutionary perspective remains underexplored.

Here, we investigated sequence evolution within the human population and across mammals for a set of alt-ORFs. These

were extracted from a recently published collection of all protein-coding ORFs, which was compiled from diverse studies of ribosome profiling—mapping ribosome coverage of RNA across the genome.

We identified the sequences of the alt-ORFs within the human genome and developed a bioinformatic pipeline to trace their evolutionary trajectories across a wide array of mammalian genomes using the Zoonomia multiple mammal coding sequence alignments. In addition to examining evolutionary origin and constraint, we explored sequence variation within the human genome by utilizing the gnomAD variant database. In contrast to the previous belief that alt-ORFs are subject to more rapid evolutionary changes, our investigation reveals that many alt-ORFs are, in fact, evolutionarily conserved.

The findings suggest that alt-ORFs as a whole are not mere genetic anomalies or "junk" translation but that some may hold crucial functions that have been overlooked in genetic research. This study provides a foundation for further exploration into the complexities of the human genome and will encourage additional research into the molecular functions of alt-ORFs in humans and across species.

907S **In space no one can hear you sweep: Investigating the dynamics and outcomes of selective sweeps in continuous geographic space** Clara Rehmann[1], Peter Ralph[2], Andrew Kern[1] [1]Institute of Ecology & Evolution, University of Oregon, [2]Institute of Ecology & Evolution, Department of Mathematics, University of Oregon

Understanding the process by which organisms adapt to their environment is a driving question in evolutionary biology. At the genetic level, positive selection, including selective sweeps, contribute significantly to this process, and in doing so, play a powerful role in shaping patterns of genetic variation. While a large body of research has explored the dynamics and consequences of selective sweeps under both theoretical and empirical frameworks, that work has focused largely on sweeps in well-mixed, randomly-mating populations. However, spatial population structure also has profound effects on how genetic variation is shared in natural populations, and the impact of this structure on selection remains uncharacterized. Here, we use forward-in-time, individual-based continuous space simulation, to investigate the dynamics of selective sweeps in spatially-structured populations. We identify and describe the relationship between selection, spatial population structure, and the movement of an adaptive variant across geographic space. Using these relationships, we leverage spatial data to identify potentially adaptive variants in Anopheles mosquito populations.

908S **Interpreting differences in DNA base composition at polymorphic sites across populations** Sheel Chandra[1], Ziyue Gao[2] [1]Biology, University of Pennsylvania, [2]Genetics, University of Pennsylvania

DNA base composition is a fundamental attribute of the genome that can vary across species but can also be surprisingly conserved between some distantly related species. Understanding the evolutionary basis of these patterns can help shed light on several related genomic features, such as codon usage, methylation, and genome organization. Recently, Li et al. (2015), reported striking differences in GC content at polymorphic sites between populations within the same species that experienced or did not experience recent bottlenecks. Specifically, individuals in bottlenecked groups showed lower GC content at common single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF) above 5% compared to those in non-bottlenecked groups across all eight species studied, including humans. These findings seemingly suggest rapid evolution and future divergence in base composition between populations.

In this study, we aim to probe the evolutionary forces underlying the pattern of base composition in human populations, using high-coverage sequencing data from the 1000 Genomes Project. We first replicate the observation of higher GC content in African individuals at common SNPs. Within each population, we also observe clear differences in the site frequency spectrum (SFS) across different mutation types (e.g., C>T, T>G), consistent with the effect of GC-biased gene conversion (gBGC). We hypothesized that gBGC, interacting with population demographic history, may have led to the inter-population difference in GC content. However, we observe higher GC content at strong-to-weak (S>W) mutations but lower GC content at weak-to-strong (W>S) mutations in African individuals. Further investigation into the SFS reveals that these differences in GC content come from the lower derived allele frequencies (DAF) in African populations for all mutation types at common SNPs, likely reflecting the impact of demographic history. However, there is an enrichment of derived alleles in African individuals relative to non-Africans, at rare SNPs (DAF<1%), the opposite pattern to common SNPs. In fact, the inter-population difference in GC content completely disappears when all common and rare variants are considered jointly. We also repeat this analysis for two other species in the original study and find similar results. We conclude that given current data, there is no evidence of differences in mutation bias or genomic GC content across human populations. Furthermore, although there is clear evidence of gBGC within populations, the strength of gBGC, combined with population demographic history, is not strong enough to produce discernible differences in base composition between recently diverged groups.

909S **Adaptive Evolution In *TAS2R14* and *TAS2R38* Bitter Taste Receptor Genes In Primates** Gillyoung Koh[1], Jiajia Chen[1], Kyra Kovacic[2], Barbara Domingues Bitarello[1] [1]Bryn Mawr College, [2]Children's Hospital of Philadelphia

Primates commonly avoid bitter-tasting foods as a potentially adaptive response to avoid ingesting toxic compounds. Bitter compounds bind to the extracellular N-terminus and the 7 transmembrane domains of type 2 receptors (TAS2R). A promiscuous receptor, the human TAS2R14 binds more than 150 bitter compounds. It modulates innate immune responses in the mouth and bronchodilation in the lungs in response to quorum-sensing molecules, is expressed in epidermal keratinocytes, and facilitates blood-cerebrospinal fluid barrier molecule transport. The human TAS2R38 binds to 23 known ligands and in humans and chimpanzees influences individual perceptions of phenylthiocarbamide (PTC), a synthetic compound structurally resembling natural chemicals found in *Brassica sp*.

Moderate frequency human *TAS2R38* haplotypes are associated with different PTC-tasting phenotypes, suggesting the action of long-term balancing selection (LTBS), while its recent evolution may be explained by demographic events alone, suggesting a complex evolutionary history. We previously reported that LTBS may have shaped human *TAS2R14* evolution. Natural selection genomic scans suggest increased immune surveillance has been a target of selection, including several genes: MHC, keratin (the first line of defense in the skin), and cell surface receptors broadly. We assess if the ability to bind a diverse repertoire of ligands has been targeted by selection. Specifically: are adaptive evolution (AE) signatures in *TAS2R14* and *TAS2R38* pervasive in primates or human-exclusive? Do specific sites display stronger AE signals? Do these sites tend to be located in specific portions of the protein? With molecular evolution models implemented in PAML (site models) and Hyphy (BUSTED, FEL, MEME), we looked at the nonsynonymous to synonymous substitution rates ratio (dN/dS) in alignments of 29 primate species (317 codons) for *TAS2R14* and 46 primate species (333 codons) for *TAS2R38*.

We find strong signals of gene-wide AE (non site-specific) (BUSTED, $p = 0.0067$ for *TAS2R38*; $p = 0.0234$ for *TAS2R14*). We also find strong AE signals for sites selected pervasively across the tree: 8 sites for *TAS2R38* and 12 for *TAS2R14* (FEL $p \leq 0.10$); 1 for *TAS2R38* and 22 for *TAS2R14* (PAML M7 vs M8, posterior $p \geq 0.90$); 21 for *TAS2R38* and 26 for *TAS2R14* (MEME $p \leq 0.10$). Work underway will assess if sites differ across the phylogeny, shedding further light on ligand binding mechanisms in these receptors and selective pressures driving AE.

910S **A scalable approach to estimating a genome-wide gene-conversion map from the UK Biobank data** Siddharth N Avadhanam, April Wei Cornell University

Gene conversion (or non-crossover recombination) involves short unidirectional transfer of DNA sequence between homologous chromosomes during meiosis. It is one of two mechanisms of homologous recombination (the other being crossover recombination) and has been shown to have a profound impact on the nucleotide composition of the human genome, yet there has been relatively less attention paid to simulating or measuring this process. Recent approaches to detecting gene-conversions in humans have relied on a three-generation genotyped pedigree study design. However, such data are difficult to obtain, are limited by SNP ascertainment biases and can only capture transmitted gene-conversions from a single meiosis, limiting our understanding of this important process. Here we propose a new approach that relies on sequence identity within a short genomic region between admixed haplotypes and a panel of reference haplotypes. We then propose an estimator of gene-conversion rate based on the coalescent process relating the sequence identity to their MRCA and time of admixture, allowing us to jointly model mutations, gene-conversions and sequencing errors. This approach allows us to capture information from every gene-conversion event between the MRCA and the admixed segment, affording higher resolution through the use of Whole Genome Sequence (WGS) data, and increasing the power to detect gene-conversion events through the use of unrelated admixed genomes. To validate our method, we built a pedigree simulator that incorporates both crossover and gene-conversion and outputs a ground truth localization of gene-conversion events; to our knowledge, this is the only available method to simulate such data. Using simulated genomes of mixed African and European ancestry, with gene-conversion events occurring only after admixture, we find that our approach is well-powered to detect these events. We are currently applying this new method to the UK Biobank data and we anticipate that the resulting genome-wide gene-conversion map will be a highly useful resource for simulating and modeling gene-conversions as well as for studying meiotic recombination.

911S **A phylogenetic approach to understand evolution and transmission of drug-resistant mutations of Human Immunodeficiency Virus Type 1 infections in a large US Kaiser Permanente Northern California clinic population** Marisol Contreras, Pleuni S. Pennings Biology, San Francisco State University

In 2022, 29.8 million Human Immunodeficiency Virus Type 1 (HIV-1) patients were on antiretroviral therapy (ART). Drug resistance to antiretrovirals is a big problem with transmitted drug resistance levels being around 10% and higher for those with prior ART exposure. Drug resistance in the virus of a patient can arise either through de novo evolution, where the patient is initially infected with a susceptible strain that later becomes resistant, or through host-to-host transmission, where the patient is infected with a strain that is already resistant to drugs. It is not well understood how often resistance is due to transmission versus de novo evolution. Host-to-host transmission of drug resistant strains leads to clusters of similarly

resistant strains in phylogenetic trees. The objective of this study is to analyze cluster sizes of drug resistance mutations on a phylogeny to understand the relative roles of de novo and transmission from a publicly available dataset of patients from Kaiser Permanente Northern California (KPNC). My plan is to use methods previously used in the Pennings lab to construct phylogenetic trees, to identify origins of resistance and clusters of resistance within ART-Naive patients to determine the relative importance of transmission and de novo evolution. We expect that mutations that are known to come with a fitness cost will appear in smaller phylogenetic clusters whereas mutations that are known to be benign for the virus will appear in larger phylogenetic clusters. Results will clarify the clinical significance of transmission clusters for different drug resistance mutations in the KPNC population, contributing to an improvement of healthcare practices and disease treatment strategies.

912S **Reimagining the data structures and algorithms for scalable whole-genome analyses** April Wei, Drew DeHaas, Ziqing Pan Cornell University

Millions of human genomes are now available through large biobanks, posing challenges for researchers to store, distribute and utilize these data. To date, biobank-scale genetic data are still encoded with tabular data structures, i.e, with rows and columns representing samples and variants along the genome. While tabular formats (e.g., VCF, PLINK, and BGEN) are supported by many bioinformatic tools, they become prohibitive to store and use as the number of genomes continue to grow. An existing alternative to the tabular format is the tree sequence format (TS) which embeds the polymorphism information in the Ancestral Recombination Graph (ARG). However, the TS format cannot easily support efficient algebraic calculations, and existing ARG inference methods are either not scalable enough for biobanks (e.g., Relate), do not compress the real genome very well (tsinfer), or lose information about polymorphisms (ARG-Needle). Given these limitations, there is an urgent demand for a new data structure that can losslessly compress the genetic data while maintaining the ability to perform different types of analyses. Here we introduce the genotype representation graph (GRG), a novel graph-based representation of biobank-scale whole-genome polymorphism data that can store the same information as phased VCFs, but with a few gigabytes (GB) as opposed to tens of terabytes (TB). This radical size reduction comes from leveraging the patterns of shared mutations among samples and encoding this information in a fully connected hierarchical graph. We developed and implemented an algorithm to infer GRG that scales to a million whole genomes. When applying our method to real data, GRG can represent the 2504 whole-genomes from 26 populations in 1000 Genomes Project using 10 GB of disk space, whereas VCF, PLINK, and TS take 800 GB, 50 GB and 23 GB, respectively. Using European genomes simulated by msprime, we find that GRG is able to store a million haploid whole genomes with 3 GB, saving 99.99% and 60% storage space compared to the VCF (33 TB) file and the simulated TS (7.5 GB) respectively. Moreover, we implemented graph traversal algorithms to compute population level first-order summary statistics that run as fast as the current state-of-the-art software. We anticipate that GRG based algorithms will further improve the scalability of higher order computation and help democratize biobank-scale data access by mitigating the associated computational burden.

913S **Two-Locus Genealogies under Isolation-by-Distance and Time** Arjun Biddanda Johns Hopkins University

Patterns of linkage disequilibrium (LD) in population genetic data have been foundational for drawing insights on the genetic basis of complex traits, meiotic recombination, and the impact of natural selection. The focus on LD in theoretical population genetics has focused on demographic models with spatial and time-stratified sampling independently, but not jointly within the same model framework. Here, I derive results for summary statistics of haplotype variation under models of temporal and spatially-stratified sampling. This includes 1) the correlation in the number of pairwise differences (π_t) observed between two sampled haplotypes and 2) the product of the LD coefficient, D, between two spatially and temporally separated samples and 3) the expected value of r^2 between spatial and temporally stratified samples. I additionally evaluate how asymmetric migration and barriers to gene flow between spatial demes alters the sign and magnitude of LD statistics. Importantly, I show how temporally stratified LD-statistics can be used to evaluate historical barriers to gene flow that may not be readily inferred from modern LD statistics alone. Finally, I explore how LD-statistics from a dataset of ~600 modern and 1,159 ancient Eurasian male X-chromosomes can be used to estimate the relative strength of barriers to gene-flow across latitude and longitudinal gradients. Overall, these results provide insight into how haplotypic patterns are jointly impacted by both spatial- and temporally population structure, and guide the development of future methods using temporally stratified data for the inference of population demographic history.

914S **Manipulating sex determination in *Caenorhabditis* tetraploids to evaluate Haldane's rule** Jonathan Harbin, Ronald E Ellis Molecular Biology, Rowan-Virtua SOM

In 1922 Haldane noted that when two species form hybrids, if "one sex is absent, rare, or sterile, that sex is the heterozygous sex." Haldane's rule applies to crosses between the closely related nematode species *C. nigoni* and *C. briggsae*, since Woodruff et al observed that crosses between *C. nigoni* males and *C. briggsae* hermaphrodites produced no living males, and that the reciprocal crosses between *C. briggsae* males and *C. nigoni* females produced only sterile males. Despite its broad applicability,

there is still lively debate about the factors that underlie Haldane's rule.

The *Caenorhabditis* sex determination cascade is initiated in the early embryo by the ratio between the number of *X* chromosomes and sets of autosomes. Disrupting this cascade by altering the sex determination pathway can drive animals to develop as male regardless of their *X:A* ratio. By manipulating sex determination and ploidy within nematodes we can study whether the hybrid heterozygous sex disadvantage is due to chromosome incompatibles (dominance theory) or male specific gene evolution (faster male theory). To that end, we adapted the Schvarzstein method for producing polyploid *Caenorhabditis* strains. Through inactivation of *rec-8* by RNA interference we produced a *C. nigoni* polyploid strain, a *C. briggsae* tetraploid strain already existed. DAPI staining confirmed that the *C. nigoni* female animals are tetraploid, and most likely *XXX; AAAA*, and the males appear to be *XX; AAAA*.

Remarkably, using these tetraploid strains, we can now produce fertile interspecies hybrids. These not only include fertile hermaphrodites, but also healthy fertile males. This result strongly supports the model that Haldane's rule in diploid crosses is caused by incompatibility between the genes on the single *X* (which of necessity comes from only a single species), and interacting products made by the pairs of autosomes from both species. As a result, negative interactions should be minimized in tetraploid males, which have one *X* from each parent species. We are now studying backcrosses of these hybrids to each parent species. Although the success rate for individual crosses is low, the resulting progeny tend to be healthy and vigorous.

Simultaneously, using a reverse genetic approach, the first *C. nigoni* sex determination mutations were generated in the critical sex genes *tra-1, tra-2*, and *fem-3*. Indels that result in an early stop codon were selected after injecting gravid *C. nigoni* females with Cas9 RNPs. Utilizing *tra-1* mutations that drive animals to develop as males, regardless of their *X: A* ratio, we are now studying if maleness can drive Haldane's rule in male *XX* hybrids.

915S **The Spatial Speedup Effect of Evolutionary and Ecological Dynamics** Anush Devadhasan, Oana Carja Carnegie Mellon University

Across scales of organization, understanding the mechanisms that shape and maintain diversity in a population remains a fundamental challenge and there exists a large literature in evolutionary biology describing the processes that can account for persistent coexistence within populations. At their core, the theoretical frameworks are grounded in balancing selection, including negative frequency-dependent (NFD) selection, spatial or temporal habitat heterogeneity, and heterozygote advantage. In particular, NFD selection, by favoring rare types, is often suggested as the main selective force for biodiversity and polymorphism in natural populations. In this talk I will describe an evolutionary model in which, unintuitively, the opposite occurs: NFD selection decreases coexistence relative to neutrality, for certain spatial population structures. We call this the spatial speedup effect and, using a combination of mathematical analysis and simulation, we find that the condition for this effect depends critically on the spatial arrangement of the population. We show that spatial speedup is specifically shaped by the algebraic connectivity of the population: a measure of "spatial bottleneckedness" that derives from spectral graph theory. I will discuss how spatial speedup in bottlenecked populations can significantly impact biodiversity over evolutionary and ecological timescales by analyzing fixation times and species richness as the respective proxies for coexistence.

916S **`diplo-locus`: A lightweight toolkit for inference and simulation of time-series genetic data under general diploid selection** Xiaoheng Cheng[1], Matthias Steinrücken[1,2] [1]Ecology & Evolution, University of Chicago, [2]Human Genetics, University of Chicago

Whole-genome time-series allele frequency data are becoming more prevalent as ancient DNA (aDNA) sequences and data from evolve-and-resequence (E&R) experiments are generated at a rapid pace. Such data presents unprecedented opportunities to elucidate the dynamics of adaptative genetic variation. However, despite many methods to infer parameters of selection models from allele frequency trajectories available in the literature, few provide user-friendly implementations for large-scale empirical applications. Here, we present `diplo-locus`, an open-source Python package that provides functionality to simulate and perform inference from time-series under the Wright-Fisher diffusion with general diploid selection. The package includes Python modules as well as command-line tools and is distributed on Python Package Index (PyPI). We demonstrate its superior computational speed compared with existing time-series methods and benchmarked its statistical performance with simulation. Application on UK samples in the Allen Ancient DNA Resources database recovered strong selection signals on rs4988235, well-established to regulate the expression of the lactase gene *LCT*. We further estimated the selection parameters of this SNP, showing evidence for its beneficial allele to be dominant. We believe this package will prove convenient and useful for future evolutionary genomics research.

917S **Spatial structure alters the site frequency spectrum produced by hitchhiking** Jiseon Min[1], Misha Gupta[2], Michael M Desai[2], Daniel B Weissman[3] [1]IE2, University of Oregon, [2]Harvard University, [3]Emory University

The reduction of genetic diversity due to genetic hitchhiking is widely used to find past selective sweeps from sequencing data, but very little is known about how spatial structure affects hitchhiking. We use mathematical modeling and simulations to find the unfolded site frequency spectrum left by hitchhiking in the genomic region of a sweep in a population occupying a 1D range. For such populations, sweeps spread as Fisher waves, rather than logistically. We find that this leaves a characteristic 3-part site frequency spectrum at loci very close to the swept locus. Very low frequencies are dominated by recent mutations that occurred after the sweep and are unaffected by hitchhiking. At moderately low frequencies, there is a transition zone primarily composed of alleles that briefly "surfed" on the wave of the sweep before falling out of the wavefront, leaving a spectrum close to that expected in well-mixed populations. However, for moderate-to-high frequencies, there is a distinctive scaling regime of the site frequency spectrum produced by alleles that drifted to fixation in the wavefront and then were carried throughout the population. For loci slightly farther away from the swept locus on the genome, recombination is much more effective at restoring diversity in 1D populations than it is in well-mixed ones. We find that these signatures of space can be strong even in apparently well-mixed populations with negligible spatial genetic differentiation, suggesting that spatial structure may frequently distort the signatures of hitchhiking in natural populations. This work has been published in Genetics (iyac139).

919S **Impacts of demographic history on inter-chromosomal haplotype phasing** Cole M Williams[1], Amy L Williams[2], Sohini Ramachandran[1] [1]Brown University, [2]23andMe, Inc.

Several recently developed methods have improved haplotype phasing by leveraging the regions of the genome that relatives share identical-by-descent (IBD). Given a focal individual F and a person R related to F through only one parent, one can phase F at positions covered by the IBD segments the pair shares (yielding inter-chromosomal phase). However, if R shares IBD segments with F through both parents (e.g., due to consanguinity at some point in the genealogy), this phasing procedure would not perform well. In the UK Biobank, the average individual has thousands of relatives, most of whom are distantly related. When conducting this IBD-based phasing in the UK Biobank, we found an abundance of relative pairs connected through both parents. Using IBD segments enables inter-chromosomal phasing but can be stymied if an individual is related to a focal sample F through both F's parents. Here we study the population genetic properties of these genealogical reticulations in the context of a population's demographic history. In a constant-sized population of N, for example, we expect two individuals' sets of ancestors to completely overlap log2(N) generations in the past. If two IBD segments date back to more than log2(N) generations in the past, their paths through the population genealogy should be independent, and the assumption that the pair is related through only one parent will frequently be violated. Thus, the frequency of relatives who are related to both parents may offer insight into the demographic history of the population, the degree of pedigree collapse, and provide guidance for IBD-based phasing. Heterogeneity between some individuals in the co-inheritance of IBD segments may offer additional insights into the degree to which random mating is violated and may help to detect population structure. We analyzed two datasets: 1029 UK Biobank samples (trio children) and genomes simulated through the Quebec BALSAC genealogy. In the UK Biobank, we find that the likelihood of a relative being related to both parents increases as relatedness decreases, such that 30% of 9th degree relative equivalents are related to both parents. In the simulated Quebec genomes, which underwent a strong founder event, the highest rate is 40% (but for 5th degree relatives) and then proceeds to decrease with decreased relatedness. These results suggest that the performance of IBD-based phasing using distant relatives depends on the dataset, and in particular the demographic history of the dataset.

920S **A population genetic model for effect size correlations in GWAS** Evan M Koch[1], Mikhail Moldovan[1], Colby Chiang[2], Shamil Sunyaev[1] [1]Biomedical Informatics, Harvard Medical School, [2]Boston Children's Hospital

The availability of well-powered genome-wide association studies (GWAS) for many complex traits has driven a large body of work on how selection shapes genetic architecture. One finding has been that GWAS loci experience negative selection that increases with the magnitude of allelic effects. What remains uncertain is how trait-level selection generates this variant-level selection. Stabilizing selection is an appealing hypothesis because it is routinely measured in natural populations and has been evidenced for some traits in modern humans. A phenotypic optimum is also suggested by symmetric negative selection between trait-increasing and decreasing alleles. However, we lack direct evidence that stabilizing selection is sufficiently widespread to explain the ubiquitous negative selection on GWAS variants. One path forward may lie in the correlation of variant effects along the genome. The Bulmer effect describes how stabilizing selection creates weak negative linkage disequilibrium (LD) between unlinked variants throughout the genome that when summed reduces the genetic variance. Recent analyses of GWAS summary statistics have found strong negative correlations at short genomic distances, suggesting a local Bulmer effect and the action of stabilizing selection. We developed a population genetics model for effect size correlations between pairs of completely linked sites using recent theory for LD between rare alleles as well as simulations. Results suggest at least two forces are qualitatively capable of generating negative effect size correlations. As expected, fitness functions resembling stabilizing selection yield negative correlations as haplotypes with opposing effects are able to drift to higher frequencies. Alternatively, when mutation is biased towards trait-increasing or decreasing effects,

symmetric fitness functions may also yield negative correlations through Hill-Robertson interference. Extending the model to consider distributions of mutational effects, we find that substantial asymmetry is necessary to generate measurable effect size correlations, but that nearly neutral mutations contribute most per-site to negative correlations under stabilizing selection. Finally, we explore extensions to pleiotropy and mutational autocorrelation. Stabilizing selection remains a plausible explanation for negative effect size correlations, but further analyses of mutational asymmetry are warranted.

921S **NovelTree: Highly parallelized phylogenomic inference** Austin H Patton[1], Feridun Mert Celebi[1], Seemay Chou[1], Jonathan Eisen[2], Megan L Hochstrasser[1], Elizabeth A McDaniel[1], Erin McGeever[1], Taylor Reiter[1], Dennis Sun[1], Ryan York[1] [1]Arcadia Science, [2]University of California, Davis

Phylogenomic analysis of thousands of gene families across diverse taxa are increasingly commonplace. However, with relatively few exceptions, implementation of complete phylogenomic analyses has been largely piecemeal, requiring that users write custom workflows that string together software implementing each each constituent step. Although such an approach allows researchers to tailor their analyses to specific use cases, this increased specificity is gained at the expense of reproducibility and comparability across studies. Furthermore, the significant computational challenges innate to phylogenomic analysis still frequently imposes practictal contraints on both the number of species, and number of gene families studied. Consequently, we developed NovelTree, a nextflow workflow designed to reproducibly conduct phylogenomic studies of gene family evolution at scale. The workflow takes proteomes (i.e. one protein amino acid sequence for each gene in a genome) from diverse organisms and infers orthology, gene family trees, species trees, and gene family evolutionary dynamics. To demonstrate NovelTree's utility for phylogenomic and comparative evolutionary inference, we applied the workflow to a dataset of 36 TSAR eukaryotes (Telonemia, Stramenopila, Alveolata, and Rhizaria).

922S **Cis-regulatory evolution of Wnt-family genes underlies morphological evolution in the domesticated silkworm** Kenta Tomihara[1], Ana Pnharanda[2], Andrew Taverner[3], Patrick F Reilly[3], Matthew L Aardema[4], Laura Kors[5], Lin Poyraz[2], Takashi Kiuchi[1], Takashi Kiuchi[1], Peter Andolfatto[2] [1]Agricultural and Environmental Biology, University of Tokyo, [2]Biological Sciences, Columbia University, [3]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [4]Ecology and Evolutionary Biology, Princeton University, [5]Biology, Barnard College

Closely-related species often exhibit distinct morphological structures that can contribute to species-specific adaptations and reproductive isolation. One example are Lepidopteran caterpillar appendages, which have evolved convergently and divergently across species. Specifically, Bombycoidea moth caterpillars evolved a distinct posterior "caudal horn" that varies in size and shape among different species within the group. To understand the genetic mechanisms responsible for the caudal horn size difference distinguishing *Bombyx mori* and its wild progenitor *B. mandarina*, we conducted a high-resolution quantitative trait locus (QTL) analysis. We detect 12 QTLs contributing to the reduced caudal horn size in *B. mori*. Notably, one QTL on chromosome 4 accounts for 12% of the mean length difference between species and encompasses a conserved cluster of Wnt-family genes, which are known to regulate morphological development in animals. By examining the expression of genes within this QTL region, we identified *Wnt1* and *Wnt6* as having caudal horn-specific cis-regulatory changes that reduce levels of expression of the *B. mori* allele. To further validate these findings, we show that CRISPR/Cas9-mediated allele-specific knockouts in F1 hybrids that reduce dosage of *Wnt1* and *Wnt6* result in a shorter caudal horn but increase body size. Despite the large contribution of *Wnt1*/*Wnt6*, our results suggest that the trait architecture is of intermediate polygenicity.

923S **Selection and introgression at sea urchin gamete recognition proteins** Matthew Glasenapp, Grant H Pogson Ecology and Evolutionary Biology, University of California, Santa Cruz

Many species of broadcast spawners exhibit species-specific fertilization mediated by gamete recognition proteins (GRPs) located on the surfaces of sperm and egg cells. GRPs often evolve rapidly under positive selection and have been implicated in establishing reproductive isolation early in speciation. However, little is known about the forms of selection responsible for GRP divergence. We incorporated data from all nine species of the strongylocentrotid sea urchin family to test for positive selection at the sea urchin GRPs bindin and EBR1, finding strong signatures of selection at both genes. Although bindin and EBR1 have been implicated in establishing reproductive isolation, both genes show signals of historical introgression, a pattern inconsistent with the hypothesis of speciation via the rapid evolution of GRPs.

924S **DNA of lizards with introgressed mtDNA shows reduced damage from Reactive Oxygen Species** Greg Haenel[1], Chase Solomon[2], Eliza Boudett[3] [1]Biology, Elon University, [2]Kennedy Krieger Institute, [3]Parexel International

Introgression of mitochondrial DNA occurs frequently where ranges of closely related species overlap and hybridization occurs. Hybrid offspring often have lower fitness due to genetic incompatibilities. Previous analyses of mitochondrial function in hybrids of the lizards *Urosaurus graciosus* and *Urosaurus ornatus* found that mitochondria from individuals with introgressed

mtDNA experienced increased rates of ATP production and oxygen consumption. Higher ATP production increases reactive oxygen species (ROS) production and high levels of ROS are one of the main causes of damage to DNA. The hybrid lizard populations have persisted and show genetic divergence so we predicted they should have mechanisms for handling higher levels of ROS in order to protect their DNA. To test this we compared levels of DNA damage from ROS in liver cells of the two parental type lizards to those of lizards with introgressed mtDNA. Isolated liver cells were treated with hydrogen peroxide, a source of ROS. Single-cell gel electrophoresis was then used to assess DNA damage. Both *U. graciosus* and *U. ornatus* tissue showed significantly higher damage than DNA from liver cells from individuals with introgressed mtDNA. We explored potential mechanisms that could explain the lower DNA damage by comparing melanin content of livers, expression levels of genes with known antioxidant function, and expression of genes with known DNA repair functions. Melanin has strong antioxidant properties but was not more concentrated in livers showing the least DNA damage. The overall gene expression profile of hybrids was very similar to *U. graciosus* and very different from *U. ornatus*, the mtDNA donor species. Seventeen antioxidant genes showed significant levels of differential expression but were not expressed consistently higher in individuals with introgressed mtDNA. SOD3 was the one antioxidant gene that had unique expression levels in hybrids. SOD2 was elevated in hybrids but not quite to the point of statistical significance. No DNA repair genes showed differential expression between *U. graciosus* and hybrids. Our results indicate that individuals with introgressed mtDNA have a mechanism operating at the cellular level that helps protect their DNA from damage by ROS. This mechanism is unknown but the superoxide dismutases, SOD2 and SOD3, warrant further investigation of their functional roles in these hybrids.

925S **Do Immune Genes Contribute to Genetic Diversity in Natural Populations? A Case Study in European Daphnia pulex** Madison Doceti, Connor Murray, Robert Porter, Alan Bergland Department of Biology, University of Virginia

The innate immune system is the first line of defense in many organisms, ranging from invertebrates to vertebrates. The Toll and Imd pathways are components of this system that target gram-negative bacteria, gram-positive bacteria, and fungal pathogens. *Daphnia pulex*, an organism critical to studying evolutionary dynamics and complex environmental interactions, contains many of the conserved proteins in these pathways. However, how the gene families of the innate immune system in *Daphnia* have changed and developed over time is unknown. In order to conduct this study, we will first identify orthologs in our species using other *Daphnia* species. Then, we will analyze the gene sequences by calculating nucleotide diversity and Tajima's D, and using the McDonald-Kreitman test. We expect that the proteins involved with pathogen recognition and defense will have diversified the most over time. Transduction proteins, however, should undergo less adaptive selection due to less interaction with outside pathogens. By obtaining these results, we can add to our understanding of coevolutionary dynamics by focusing on how the immune system adapts to its environment. Further, we will explain how changing environments can affect this adaptability, which may explain how immune genes contribute to diversity in natural populations.

926S **High-resolution mapping of recombination events in a vertebrate species lacking PRDM9, the zebra finch (*Taeniopygia guttata*)** Djivan Prentout, Marc de Manuel, Carla Hoge, Molly Przeworski Columbia University

In vertebrates, meiotic recombination is concentrated in short regions of 1-2 kb, known as hotspots. In many vertebrates, including mice and humans, a protein called PRDM9 directs recombination to the genome by making two histone modifications (H3K4me3 and H3K36me3). In species lacking PRDM9, such as canids and birds, little is known about the underlying mechanism, but analyses of linkage disequilibrium data indicate that historical recombination rates are increased near promoter-like features (notably, transcription start sites and CpG islands). To learn more about properties of recombination in the absence of PRDM9, we focused on the zebra finch (*Taeniopygia guttata*), a species with an excellent assembly, and resequenced whole genomes from three-generation pedigrees (totaling 54 meiosis). Given the high nucleotide diversity within the species (~1%), this approach allowed us to resolve ~1100 crossovers (CO) events within a median interval length of 650 bp and to call ~500 non-crossover (NCO) events. Overall, there is no significant difference in rates of CO or NCO between sexes (sex-average rate = 2.28 cM/Mb). Crossovers are enriched in telomeric regions of macrochromosomes, whereas non-crossover events appear to be more uniformly distributed. Both recombination resolutions are enriched near promoter-like regions, suggesting that double strand break rates are indeed increased near these features. Focusing on NCO, we estimate that conversion tracts of NCO events are on average only 27 bp, similar to estimates in mice. In turn, while we see evidence for GC-biased gene conversion, the strength appears to be slightly lower than what has been reported for mice. More generally, our approach illustrates how the analysis of a relatively small number of pedigrees in a species with high nucleotide diversity can help to characterize basic parameters of recombination.

927S **Beyond Resistance: Tracing the Ancient Threads of Antibiotic Evolution and Their Impact on Microbial Genomes** Alejandro Marcos Gil Gomez Ecology and Evolution, Stony Brook University

Antibiotic resistance has become a pressing biomedical challenge in the twenty-first century, yet its origins trace back billions of years when soil microorganisms evolved resistance to natural compounds. This evolutionary phenomenon, paralleling the development of natural antibiotics, is exemplified by Streptomyces bacteria, responsible for over two-thirds of modern

antibiotics. These bacteria employ biosynthetic gene clusters (BGCs) in their genomes, encoding enzymes, efflux pumps, and self-resistance genes crucial for antibiotic production.

This study explores the hypothesis that the evolution of antibiotics has exerted a profound influence on the rate of evolution of antibiotic targets and prompted compositional changes in bacterial genomes. Aminocoumarins and aminoglycosides, two classes of natural antibiotics, serve as focal points for the investigation. I employed phylogenetic reconciliation techniques, utilizing time-calibrated species trees of antibiotic-producing organisms and gene trees for BGC genes. These gene trees are reconciled to the species tree, providing dating estimates for the most recent common ancestor of each gene and thus offering insights into the origin of aminocoumarin and aminoglycoside antibiotics.

To test the hypotheses, the study explores shifts in the evolutionary rates of antibiotic targets by comparing the distribution of evolutionary rates of branches that split before and after the evolution of antibiotics.

The results indicate that the emergence of both aminocoumarin and aminoglycoside BGCs triggered a significant shift in selective forces, influencing both the evolutionary rates of their targets. This evolutionary transition, dating back hundreds of millions of years, represents a landmark event in the trajectory of microbial life. Understanding the far-reaching consequences of this major evolutionary shift is crucial for predicting the impact of new synthetic antibiotics on global evolutionary patterns in pathogens and other microbes. This research contributes valuable insights into the intricate interplay between antibiotics, microbial evolution, and the broader implications for global health.

928S **Gene and Dental Morphology Association** Julia Aloi[1], Wynn Meyer[2], Michael Tene[1], Matthew Pollard[3], Emily Puckett[3] [1]Biological Sciences, Lehigh University, [2]Lehigh University, [3]University of Memphis

Dental morphology – the study of the size, shape, and pattern of teeth – has been used to identify similarities in dental traits across different mammalian lineages, providing key insights into diet patterns. Teeth morphology can additionally be understood within the realm of convergent evolution. This occurs when, despite having distinct evolutionary histories, species may independently evolve similar dental features to adapt to their specific dietary habits and ecological niches. Understanding these convergent patterns is crucial for deciphering the underlying principles governing dental morphology in mammals.

Our research explores the relationship between genetic evolution and dental morphology in mammals by investigating the correlation between the relative evolutionary rates of genes and the evolution of convergent binary or continuous dental morphological traits across a phylogeny. This endeavor focuses on the relationship between genetic factors and the development of dental patterns in mammals, providing valuable insights into the evolutionary processes shaping their diverse dental morphologies.

To achieve this goal, we used the Relative Evolutionary Rate Convergence (RERConverge) method to quantify the association between the evolutionary rates of conserved non-coding elements and dental morphological traits. For dental morphology, we collected raw dental measurements and calculated metrics including Orientation Patch Count (OPCR) and Dental Morphological Trait Analysis (DMTA). These datasets cover a broad spectrum of mammalian species, both extinct and extant, enabling a comprehensive analysis of dental morphology across evolutionary time scales. For genetic data, non-coding regions[2] near tooth development genes taken from Zoonomia's mammalian genetic dataset were used.

By associating the evolutionary rates of specific non-coding elements to the convergent evolution observed in dental features, we focus on the potential influence of genetic factors on the development and adaptation of mammalian dental patterns. Through the analysis of diverse datasets and the application of RERConverge, we analyze the interplay between genetic evolution and the convergent development of dental traits in mammals. This knowledge holds the potential to enhance future understanding of broader evolutionary processes and the adaptations that have shaped the diversity of mammalian dental morphology.

929S **Developing a speciation model derived from polygenic epistasis** Emmanuel D›Agostino[1], Diogo Melo[1], Julien Ayroles[2,3] [1]Princeton University, [2]Ecology and Evolutionary Biology, Princeton University, [3]Lewis-Sigler Institute of Integrative Genomics, Princeton University

The Dobzhansky-Muller incompatibility (DMI) model of speciation, in which postzygotic isolation arises from deleterious epistatic interactions in hybrid genomes, has held up for over a century. However, the recent finding that weak deleterious epistasis is widespread within species calls into question exactly how DMI speciation proceeds: can many small-effect interactions between not-yet-fixed alleles reduce gene flow between populations enough to reproductively isolate them? Using forward-genetic simulation, we compare scenarios in which many weak incompatibilities drive divergence to those

in which fewer, stronger ones do, and measure the accrual of reproductive isolation and genetic differentiation as well as the maintenance of DMI alleles. We also explore the relative contributions of drift and selection to these models by varying population sizes, the marginal and epistatic effects of incompatible alleles, and the initial distributions of these alleles in populations after divergence. Our results show that many small-effect DMIs can lead to the accumulation of divergence about as rapidly as fewer, larger ones, in some cases. Specifically, non-homogeneous initial distributions of DMI alleles and larger population sizes were among the factors that facilitated many weak DMIs driving comparable divergence to few strong ones. Taken together, this work explores the cases in which negative fitness epistasis can be indicative of a path to postzygotic reproductive isolation and speciation outside of the large-effect "speciation gene" paradigm, and some of the parameter spaces in which it occurs.

930S **Variation Graph Pangenomes Improve Read Mapping and SNP Calling Accuracy in Divergent Diploid And Allopolyploid Populations** Justin Conover[1], Ryan N Gutenkunst[2], Michael S Barker[3] [1]University of Arizona, [2]Molecular and Cellular Biology, University of Arizona, [3]Ecology and Evolutionary Biology, University of Arizona

A fundamental first step in population genomic analyses is choosing a reference genome to map sequencing data against for variant discovery. When considering an analysis of divergent populations or species, the population that is more distantly related to the reference genome will typically have less accurate variant discovery than the more closely related population, a phenomenon known as "reference bias". Allotetraploid genomes present a unique bioinformatic challenge. These polyploid genomes are composed of two complete chromosomal complements from two divergent progenitor species. Hence, using either diploid progenitor's genome as the reference will necessarily lead to a reference bias for variants in the subgenome from the other parental species. Additionally, recombination between duplicated homologous chromosomes (homoeologous exchange) can alter the dosage expectation of a given chromosomal region, further complicating variant discovery in allotetraploids. Here, we explore reference biases in a population of allotetraploid Brassica napus and its diploid progenitors, B. rapa and B. oleracea. We find that although reference biases abound when mapping to the reference genome of either diploid reference, these biases are largely ameliorated by creating and mapping reads to a variation graph pangenome composed of both diploid reference genomes. We also find that the method used to construct the variation graph pangenome, and the variant discovery pipeline, have significant effects on the accuracy of variant discovery.

931S **Common segregation of clonal seed production and polyploidy in diploid wild plants** Yvonne Willi[1], Jana Flury[2] [1]Environmetnal Sciences, University of Basel, [2]University of Basel

Some angiosperm plant genera and families have been renowned for containing species with a diversity of clonal and sexual reproductive systems. Clonal reproduction can include the growth of secondary roots from shoots or secondary shoots from roots, and the clonal production of seeds, termed apomixis. The latter sometimes depends on sexual mating, as apomictic seed and embryo development can require the stimulus of pollination, which may be easily achieved by self-pollination. For the combined shift to apomixis and the breakdown of self-incompatibility, polyploidization has been considered the ultimate trigger, and indeed, the syndrome of polyploidy, self-compatibility and apomixis has been confirmed in a number of larger plant taxa.

Our research suggests that the syndrome, or parts of it, are more common than thought so far. We selected 8 crucifer species that were previously described as diploids and non-apomicts. We Illumina paired-end-sequenced 10 individuals of 3 or 6 populations of each species and estimated ploidy, the mating system, and the occurrence of apomixis. We found that species were either predominantly outcrossing or selfing. The two outcrossing species were diploid, with the exception of one individual that was tetraploid, and they showed no signs of apomixis. The 6 self-compatible species contained a few to a considerable number of polyploid individuals per population. Furthermore, 4 of the selfing species had some populations with a high fraction of detected apomicts. Our work suggests that the segregation of polyploids and apomicts in otherwise diploid and sexually reproducing plant species has probably been greatly underestimated. Individual-level whole-genome sequence data allows for the easy detection of polyploids and clonals, and can serve as the starting point for the study of the adaptive significance of this diversity of reproductive and genetic systems.

932S **Novel mechanisms and evolutionary dynamics of metacaspase-dependent apoptosis** Darren K Lam, Gavin Sherlock Department of Genetics, Stanford University

Apoptosis in unicellular species results exclusively in organismal death. Nonetheless, apoptosis is conserved across Bacteria and Eukarya, which has driven long-standing questions about whether apoptosis plays an adaptive role in unicellular species. Moreover, the mechanisms underlying unicellular apoptosis remain poorly understood. Even in S. cerevisiae, one of the best-studied model organisms for unicellular programmed cell death, many components of the apoptosis pathway(s) remain unidentified. To this end, I have performed multiple sequence alignment on the known apoptotic machinery in yeast. My preliminary data suggest the evolution of a complex regulatory mechanism by which the yeast metacaspase Yca1 initiates

apoptosis based on the level of cellular proteotoxicity; I am currently developing an optical assay to evaluate this hypothesis. In addition, I have designed a set of CRISPRi and CRISPRa screens to provide an orthogonal bottom-up approach for the characterization of a novel metacaspase-dependent apoptosis pathway. Finally, to address whether apoptosis plays an adaptive role in unicellular species, I will evolve barcoded yeast libraries and directly measure the impact of metacaspase-dependent apoptosis on long-term population fitness.

933S **The evolution of recombination rate modifiers during selection for complex traits** Enrique Schwarzkopf, Nathan Brandt, Caiti Smukowski Heil Biological Sciences, North Carolina State University

Recombination rates vary between populations and species across the tree of life. It has been observed that domesticated plant varieties often have higher recombination rates than their wild congeners. As such, artificial selection on traits of interest appears to indirectly select for higher recombination rates. Variants of genes that increase overall recombination rates–referred to as recombination rate modifiers–are expected to increase fitness under certain forms of selection (e.g., fluctuating selection or selection on multi-locus traits). We will use a panel of outbred populations of *Saccharomyces cerevisiae* experimentally evolved under different strengths of selection (which show evidence of selection at different sets of loci) to explore how different forms of selection affect recombination rates. We will also use evolutionary simulations in SLiM to expand the parameter space of possible strengths of selection and sets of loci under selection to gain further insight into how recombination rates are modified.

934S **Comparing Carnivorous and Herbivorous Mammalian Serine Dehydratases by Growth Rate in Saccharomyces cerevisiae** Julia Adamowicz[1], Michael Tene[1], Helberth Quisbert[2], Greg Lang[1], Cal Shutack[1], Wynn Meyer[1] [1]Lehigh University, [2]St. Peter's University

Convergent evolution is a biological process in which independent lineages experience natural selection that leads to the development of similar traits or phenotypes. This phenomenon generates distinctive molecular patterns in the genetic makeup of organisms facing similar selective pressures. In prior work, we used convergent evolution to connect genes with phenotypes. In this study, we functionally verify proteins we associated with mammalian carnivory/herbivory differentiation using Saccharomyces cerevisiae . Specifically, we are focusing on the genes responsible for serine/threonine catabolism, SDS (Serine Dehydratase) gene and SDSL (Serine Dehydratase-like) genes, and the yeast homologue of both genes cha1. Our prior work determined that the geneset with the strongest connection to diet was the genes involved in branched chain amino acid metabolism. To assess the in-vivo functionality of the orthologs, we measured the ability of the ortholog to metabolize serine/threonine using S. cerevisiae. For this, we selected a preliminary set of orthologs from carnivorous and herbivorous mammals. These were chosen by selecting a pair of closely related carnivore and herbivore clades, and selecting a representative [elaborate] species from within each clade.We designed plasmids to replace the yeast homologue cha1 with our chosen mammalian orthologs of SDS or SDSL, enabling their expression in yeast. We anticipate observing growth rate differences between carnivore genes and herbivore genes in conditions where the enzyme's substrate is the primary nitrogen source. Specifically, we expect Carnivore-cha1 to exhibit increased growth on Serine and Threonine as a nitrogen source relative to Herbivore-cha1. We expect differences in growth rates between carnivores and herbivores, which will serve as compelling evidence of the impact of convergent selection and further validate the computational findings.

935S **Experimental evolution of biofilms in environmental isolates of the budding yeast *Saccharomyces cerevisiae*** Jennifer Lin[1], Despina Mason[1], Benjamin Epley[2], Helen Murphy[3] [1]Biology, William & Mary, [2]Ecology & Evolution, University of Chicago, [3]William and Mary

In environmental and clinical settings, many microbes form biofilms. These are communities in which cells produce an extracellular matrix, adhere to a surface and one another, and are resistant to environmental stressors. Biofilms and other adherence phenotypes are associated with pathogenic microbes, including fungal pathogens, because these traits allow them to persist in the face of insults, such as antibiotics. Opportunistic fungal pathogens that normally live in the environment, but are capable of infecting hosts when presented with one, are of increasing concern [1]. To investigate the process of a fungal environmental isolate becoming a potentially pathogenic one, we evolved replicate populations of *Saccharomyces cerevisiae* under selection for adherence to a plastic surface. Isogenic colonies from three different backgrounds (oak isolate YPS681, wine isolate L-1528, and lab isolate SK1) were used to initiate replicate populations. Experimental populations were grown in the presence of a plastic bead and only those cells that adhered to the bead were used to inoculate the next growth tube, while control populations were grown without beads; half of the populations were punctuated with sexual reproduction. After 600 generations, plastic adherence increased by at least one order of magnitude in all experimental populations, despite the different genetic backgrounds beginning with different adherence abilities. Overall, sexual populations evolved at a faster rate than asexual populations. At the end of the experiment, individual clones were isolated from the final timepoint of all replicate populations and assayed for other adherence phenotypes. All genetic backgrounds had at least some final clones that

produced biofilm colonies, grew invasively in agar, and/or formed floating mats (flors). Thus, selection on plastic adherence led to the incidental evolution of other biofilm-related phenotypes. Finally, populations were sequenced at timepoints throughout the experiment and the dynamics of the mutations that accrued during the experiment were characterized. Our results demonstrate that fungal sexual reproduction can contribute to the accelerated phenotypic and genetic evolution of a of virulence-related trait, that adherence traits can be correlated, and that environmental isolates can evolve pathogenic traits in a relatively short time period.

[1] (2022) World Health Organization fungal priority pathogens list to guide research, development and public health action.

936S **Hybrid genetic analysis reveals large chromosomal effects on thermal divergence in the *Saccharomyces* species** Nilima Walunjkar, Lydia Levesque, Justin Fay Biology, University of Rochester

Thermal adaptation is universal across life and organisms have colonized a wide variety of temperature niches. Understanding the genetic basis of thermal range shifts is challenging due to the long evolutionary timescales over which they have occurred. High divergence between species with distinct thermal profiles impedes genetic mapping, as interspecies hybrids are either inviable or sterile. To address this limitation, we used a combination of novel hybrid genetic approaches.

In the *Saccharomyces* yeast species, *S. cerevisiae* and *S. uvarum* have diverged for 15 million years and differ by 8°C in their upper thermal growth limits. Interspecies hybrids are sterile and inherit thermotolerance from *S. cerevisiae*. We generated aneuploid hybrids, each missing a single *S. cerevisiae* chromosome, and found multiple chromosomes are necessary but not sufficient for thermotolerance as well as multiple chromosomes without large effects on thermotolerance. In *S. kudriavzevii*, another thermally sensitive species, all the chromosomes tested had a large effect on thermotolerance, suggesting an independent and parallel loss of thermotolerance in *S. uvarum* and *S. kudriavzevii* lineages. We validated one chromosome (chr5) in the *S. cerevisiae* – *S. uvarum* background, through reciprocal chromosome aneuploidy and used CRISPR-generated loss of heterozygosity (LOH) to fine map thermotolerance to both arms of *S. cerevisiae* chromosome 5. We also observe a gain in low temperature growth as a consequence of loss of heterozygosity in chromosome 5, indicating a tradeoff between thermotolerance and cold temperature growth.

Our results support a model of multiple genes of large effect that together are required for thermotolerance but do not exclude the possibility that each gene evolved through multiple changes over long evolutionary time scales.

937S **Mutation rate and spectrum evolution in *Saccharomyces*** Pengyao Jiang[1,2], Vidha Sudhesh[2], Megan M. Phan[2], Ishan Bansal[2], Alan J Herr[3], Maitreya J Dunham[2], Kelley Harris[2] [1]Center for Mechanisms of Evolution, Arizona State University, [2]Genome Sciences, University of Washington, [3]Department of Laboratory Medicine and Pathology, University of Washington

Mutations are the source of genetic variation and a prerequisite for evolution. Despite their fundamental importance, their rarity makes them expensive to detect and difficult to study. To address this limitation, we leverage natural polymorphisms, which are historical mutations that passed the sieve of evolution, as a tool for detecting the change of mutational process in evolutionary history. We performed PCA analysis on the mutation spectra from natural polymorphisms on a few species of Saccharomyces yeasts, chosen for extensive prior population genomic and ecological knowledge, genome engineering, and amenability for controlled lab studies. We found the most evident difference being the domestication state, rather than the phylogenetic distance as previously observed in mammals. Intriguingly, strains from *S. cerevisiae* African beer population exhibited a conspicuous pattern in mutation spectrum, in the opposite direction from other strains, even surpassing some inter-species mutation spectrum variations. Notably, a subset of French dairy strains displayed intermediate mutation spectra, and further *in silico* analysis is consistent with the presence of mutator alleles for A>C mutations introgressed from African beer strains. To test this hypothesis, we integrated a reporter into the genomes of several African beer and French dairy strains to be able to perform the modified fluctuation assays. Our *de novo* mutation spectra in these strains are in support of this hypothesis, with a very mild mutator effect. *De novo* mutations also surprisingly exhibited greater variation in mutation types beyond A>C, suggesting that mild mutators are more likely to preserve during evolution than strong ones. In summary, our study reveals patterns and processes of mutation rate and spectrum evolution in natural populations of budding yeast.

938S **Living with a killer: how coevolved *Saccharomyces cerevisiae* become toxin resistant** Michelle Hays[1], Angelina Chan[1], Magdalena Pieczynska[2], Arjan De Visser[3], Gavin Sherlock[1] [1]Genetics, Stanford University, [2]University of Warsaw, [3]Wageningen University

Some yeasts are killers. They secrete toxins that kill neighboring cells, but protect themselves with an intracellular antidote. Killers have an advantage in mixed populations, although toxin production comes at a metabolic cost. Many *Saccharomyces cerevisiae* strains require two viral genomes to be killers. Along with sensitive cells in the environment, this is a complex

genetic conflict: yeast and viral genomes alike are capable of adaptation and fitness tradeoffs abound at many levels. Our research dissects the molecular basis of adaptation in the face of these competing selection pressures to understand evolutionary outcomes.

We identified beneficial mutations that arose in coevolved killer and sensitive yeast, including mutations responsible for toxin-resistance. Through bulk segregant analysis, we identified a putative gain-of-function missense mutation in the HOG osmoregulatory pathway component *SSK1*. Preliminary data suggest this dominant-acting polymorphism is sufficient to protect yeast from the coevolved toxin, and other killer toxins. Ongoing experiments aim to determine the mechanism by which this polymorphism creates toxin-resistance and any host tradeoffs associated with the coevolved allele. Preliminary data suggest this mutation may affect protein-protein interactions, leading to impacts on both HOG and Cell Wall Integrity (CWI) pathways. This mutation is fit under laboratory coevolution conditions, but falls in a domain that is strictly conserved in natural isolates. *SSK1* is required for multiple drug resistance in *Candida auris*, and our ongoing work will address whether this gain-of-function allele might be present in fungi associated with human pathogenicity and drug resistance. In future, we aim to further understand how killer yeasts counter-adapt in the face of these resistant competitors, and how viral genomes evolve as competing host population composition changes.

939V **Navigating the genomic diversity landscape of the hyper-polymorphic nematode *Caenorhabditis brenneri*: insights and challenges** Anastasia Teterina[1], John H Willis[1], Murillo F Rodrigues[1], Angel G Rivera-Colón[1], Peter L Ralph[1,2], Andrew D Kern[1], Patrick C Phillips[1] [1]Institute of Ecology and Evolution, University of Oregon, [2]Department of Mathematics, University of Oregon

Patterns of genetic variation carry footprints of population history and evolution of the species. However, it is challenging to decode these signals and dissect what events and forces have shaped the diversity landscape. This task becomes even more complex for species with high levels of genetic variation, including structural, as most of the standard population genetic approaches use only diallelic single nucleotide variants and exclude all other variation. What are the implications of such bioinformatic choices on inference of population processes in hyper-polymorphic species? In the era of haplotype-resolved individual genomes, how can we enhance our approaches to studying population processes?

Caenorhabditis brenneri is an outcrossing species of nematodes in the ‹Elegans› subgroup (Rhabditida; Sudhaus & Kiontke 2007), which is currently known to be one of the most genetically diverse eukaryotes, with nearly one in ten nucleotides being polymorphic, making its population diversity level comparable to that of bacteria (Dey et al. 2013). We generated a gapless reference genome for *C. brenneri* and sequenced several individual nematodes from different parts of its range using long reads (PacBio HiFi). We employed and compared various bioinformatic approaches, including standard mapping to the reference, variation graphs, and alignment of individual assemblies to analyze the genetic variation and structural variation landscapes in *C. brenneri*. Additionally, we tested with simulations how the exclusion of multiallelic sites can affect the inference of population history and estimation of diversity for populations with different levels of genetic variation. Our results denote a potential step forward in achieving a more comprehensive analysis of high-quality population data for highly polymorphic species, contribute to a deeper understanding of the population history of *C. brenneri*, and enable more precise estimations of genome-wide diversity patterns within its population.

940V **Evolutionary graph theory beyond single mutation dynamics: on how network structured populations cross fitness landscapes** Yang Kuo[1], Oana Carja[2] [1]Computational biology, Carnegie Mellon, [2]Carnegie Mellon

Spatially-resolved datasets are revolutionizing knowledge in molecular biology, yet are under-utilized for questions in evolutionary biology. To gain insight from these large-scale datasets of spatial organization, we need mathematical representations and modeling techniques that can both capture their complexity, but also allow for mathematical tractability. Evolutionary graph theory utilizes the mathematical representation of networks as a proxy for heterogeneous population structure and has started to reshape our understanding of how spatial structure can direct evolutionary dynamics. However, previous results are derived for the case of a single new mutation appearing in the population and the role of network structure in shaping fitness landscape crossing is still poorly understood. In my talk, I will study how network structured populations cross fitness landscapes and show that even a simple extension to a two-mutational landscape can exhibit complex evolutionary dynamics that cannot be predicted using previous single-mutation results. I will show how our results can be intuitively understood through the lens of the two main evolutionary properties of a network, the amplification and deceleration factors, and further discuss how to link these models to spatially-resolved datasets of cellular organization.

941V **Rapid changes in the recombination landscape shape patterns of genetic diversity and molecular evolution in *Drosophila*** Ana Pinharanda[1], Sheel Chandra[1], Kevin Deitz[1], Lucas Hemmer[2], Mitra Kardestuncer[3], Young Mi Kwon[1], Naima Okami[1], Julie Peng[4], Patrick Reilly[4], David Stern[3], Justin Blumensteil[5], Peter Andolfatto[1] [1]Columbia University, [2]University of Kansas, [3]The University of Chicago, [4]Princeton University, [5]Howard Hughes Medical Institute

Recombination shapes genomic diversity by determining patterns of linkage disequilibrium and the degree to which selected sites impact the evolutionary dynamics at linked sites. Despite its importance, we are only beginning to appreciate the extent to which broad-scale recombination rates vary between closely-related species and how that may influence species-specific patterns of genomic variation and molecular evolution. Here, we construct detailed genetic maps by individually genotyping over 16 thousand recombinant flies from three species in the *Drosophila melanogaster* species complex (*D. simulans*, *D. yakuba*, and *D. teissieri*) and compare them to an existing high-resolution map for *D. melanogaster*. While autosomal recombination rates remain broadly conserved between species, we identify big differences in the recombination landscape of the X chromosome and the extent of centromeric suppression of recombination in the *D. yakuba* lineage. Our findings highlight how the rapid evolution of the genomic recombination landscape influences lineage specific patterns of molecular evolution and adaptation.

942V **Long-Term Stability in Genomic Clines in Natural North American Populations of Drosophila melanogaster** Vitoria Horvath Miranda[1], Murillo F. Rodrigues[2], Rodrigo Cogni[3] [1]Genetics, University of Sao Paulo - Institute of Biosciences (IB-USP), [2]Institute of Ecology and Evolution - University of Oregon, [3]Ecology, University of Sao Paulo - Institute of Biosciences (IB-USP)

Sequencing and comparing historical and recent samples is a fruitful approach in the study of evolution, and it can be used to investigate possible evolutionary responses to climate change. Similarly, sampling spatially distributed populations, enables us to find links between genotypic and phenotypic variants to environmental variables. Clines are the correlation of measurable genotypic or phenotypic traits with a geographic gradient, such as latitude or altitude. *Drosophila melanogaster* displays many clinal traits, such as body size and melanization, and it is an ideal system to study the evolutionary consequences of climate change. We sequenced samples of *Drosophila melanogaster* natural populations spread along the east coast of the United States from two different time points: 1997, and 2009/2010. Additionally, we also sampled two North American populations in 2017 and 2022. In total, 16 populations were sequenced with pool-seq, SNPs were called and annotated, and their frequencies were computed. To identify clinal SNPs, we ran a generalized linear model with a binomial link function of allele frequency against latitude for each site in each period. We compared clinal SNPs of 1997 and 2009/2010 with SNPs that systematically changed between 1997 and 2022 in the northern populations. We estimated the frequencies of all seven cosmopolitan inversions to investigate their clinality. There are fewer clinal SNPs in 2009/2010 samples than in 1997. Most of this difference is driven by the reduction in south samples› frequencies of the *In(3R)Payne* chromosomal inversion. This reduction is intriguing since the increase of this inversion between 1979 and 2004 in the Australian latitudinal cline was credited to global warming. Other inversions were not clinal in either period or remained stable throughout the years. We also sequenced isochromosomal lines for the second and third chromosome prepared with three populations of 1997. With those samples, we were able to look for signs of selection and ran admixture analyses using African and European *D. melanogaster* populations as reference.

943T **Natural variation in C. elegans responses to per- and polyfluoroalkyl substance (PFAS) pollutants** Tess Leuthner, Ryan Baugh Department of Biology, Duke University

An estimated 200 million US residents are drinking water contaminated with per- and polyfluoroalkyl substances (PFAS) and 99% of all human blood serum samples tested in the US contain PFAS. Epidemiological evidence suggests that exposures to these “forever chemicals” are associated with major diseases, including cancer. However, the mechanisms of toxicity of practically all the >12,000 emerging, structurally complex PFAS remain entirely unknown. Therefore, we leveraged natural genetic variation of wild *C. elegans* to identify structure-specific molecular mechanisms of PFAS toxicity. We are investigating toxicity of ten PFAS chemicals that vary in three structural attributes (chain length, functional group, and chain composition) and the contribution of natural genetic variation on response to PFAS exposures. We first conducted a 48 hr toxicity assay in the laboratory-adapted reference strain, N2, to determine the effective concentration in which we observed a 50% reduction in growth (EC50). There was significant variation in EC50 values among PFAS chemicals which was driven by specific structural attributes of PFAS. We repeated this toxicity assay using 11 genotypically different strains from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR). We observed variation in toxicity (EC50 values) among strains within PFAS chemicals, suggesting that underlying genetic variation causes variation in response to exposures. Furthermore, we observe variation among wild *C. elegans* strains between chemicals that vary by specific molecular structures, which suggests specific gene-environment interactions in response to PFAS exposures. Next, I will pool a population of 192 naturally diverse *C. elegans* strains, expose to PFAS, and sequence the population, applying an approach developed in our lab (MIP-Seq, Webster et al. 2022) to identify sensitive and resistance strains and quantitative trait loci (QTL). I will then use various genetic and genome-editing approaches to explicitly identify the role of one or more gene variants that contribute to structure-specific toxicity of PFAS chemicals. Overall, this approach demonstrates the power of using genetics to investigate molecular mechanisms of toxicity and will result in a better understanding of variation in susceptibility to environmental contaminants.

944T **The genetic architecture of transposable element-mediated heterochromatin formation in *Drosophila***

melanogaster Kayla Ly, Yuheng Huang, Anthony D Long, Grace YC Lee Ecology and Evolutionary Biology, University of California, Irvine

Transposable elements (TEs) are mobile genetic elements capable of replicating within eukaryotic genomes, often at the expense of host fitness. Host genomes employ small RNA-based mechanisms to silence euchromatic TEs, typically through the enrichment of repressive epigenetic marks, such as H3K9me2/3. Although this mode of silencing works to inhibit TE propagation, the resultant epigenetic modifications can "spread" up to 20kb from the TE insertion site, potentially silencing neighboring genes. The enrichment of these repressive epigenetic marks around TE insertions in the euchromatic genome mirrors the well-known spreading of repressive marks from constitutive heterochromatin. However, it remains unclear which genes harbor natural variation that influences the extent and magnitude of this mark-spreading at euchromatic TEs. To unbiasedly address this knowledge gap, we use extreme-QTL mapping (X-QTL) to identify genetic loci contributing to varying spreading of repressive marks from silenced euchromatic TEs. X-QTL powerfully combines traditional QTL mapping with bulk segregant analysis, in which individuals displaying extreme phenotypes in synthetic multiparent populations are pooled and sequenced to identify causal variants. Our initial experiment demonstrated that there is variation segregating among the Drosophila Synthetic Population Resource (DSPR) founders that drives differences in mCherry fluorescence intensity from a reporter construct sensitive to TE-mediated repressive marks. This finding has propelled our current work to perform X-QTL on an outbred population of DSPR recombinant inbred lines (RILS) by similarly crossing our reporter strains to the mapping population and obtaining F1 individuals with extreme phenotypes of interest. Importantly, we have established experimental protocols for conducting large-scale crosses and implementing imaging techniques to select individuals displaying extreme levels of TE-silencing, including both weak and strong cases. Our X-QTL mapping approach will allow for the identification of previously unknown genes contributing to TE-mediated enrichment of repressive marks, and increase our understanding of natural variation in host regulatory mechanisms pertaining to selfish DNA elements.

945T **Dissecting the genetic basis of zinc toxicity resistance in *Drosophila melanogaster* using an extreme QTL (XQTL) mapping approach** Katherine M Hanson[1], Anthony D Long[2], Stuart J Macdonald[1] [1]Molecular Biosciences, University of Kansas, [2]Ecology and Evolutionary Biology, University of California

Human exposure to toxic levels of various heavy metals has risen due to environmental contamination resulting from mining, agricultural and industrial practices. Heavy metal toxicity can damage cells by damaging DNA via reactive oxygen species and oxidative stress, altering the balance of essential elements and affecting enzymatic activity. However, heavy metals like zinc are essential and it is critical appropriate levels are maintained. Zinc toxicity has been demonstrated to be a complex, polygenic trait, but has primarily been studied via traditional genetic knockdown studies which have limited genetic backgrounds. Our goal is to identify genes segregating for allelic variation for zinc resistance using an unbiased genome wide mapping approach. *Drosophila melanogaster* is an excellent model to study zinc resistance since it possesses orthologs of critical zinc homeostasis genes such as *MTF-1* (a metal response transcription factor) and has previously been successfully used to model a range of heavy metal response traits. To genetically dissect zinc resistance, we employed extreme QTL (XQTL) mapping, a powerful technique that bulk selects and sequences pools of outbred individuals with extreme phenotypes. Our outbred population was derived from the 8 *Drosophila* Synthetic Population Resource Population A founder lines, and we raised 12 large cohorts from this population on media supplemented with a toxic level of zinc, sequencing pools of emerging, zinc resistant females and matching controls. Using the known founder sequences, we estimated the founder composition for each pooled sample for windows along the genome and identified 7 QTL with significant allele frequency shifts between control and selected pools. Our QTL harbor 629 genes and 24 of these were assessed as strong causal candidate genes based on existing data. We tested 18 of these candidates for impacts on developmental zinc resistance via midgut specific RNAi by measuring emergence (the percent of eggs that reach adulthood) and development time. Knockdown of 11 of our genes impacted one or both of our phenotypes and this included *MTF-1*, *pHCl-2* (produces a zinc sensor protein) and *Mekk1* (a gene implicated in resistance to other heavy metals). Additionally, we used RNAseq to characterize regulatory changes underpinning zinc resistance. We used zinc selected and control populations developed via the same approach employed for XQTL and profiled larvae from both populations following 20-hour exposure to zinc or control media. We identified over 1200 genes whose expression change due to zinc significantly differed between the zinc selected and control populations; 54 of these genes were within our QTL intervals and included 2 of our candidates. Using a variety of techniques and genetic backgrounds, our work highlights both recognized and novel contributors to zinc resistance, and possibly resistance to other heavy metals in flies.

946T **Comparing Methods and Strategies for Complex Trait Prediction from Gene Expression** Noah K Klimkowski Arango, Fabio Morgante Department of Genetics & Biochemistry, Clemson University

Accurate prediction of complex traits is an important task in quantitative genetics. While prediction of complex traits has been pioneered in agriculture for selection purposes, it has become important in human genetics as well, in the context of personalized medicine. Genotypes have traditionally been used for trait prediction using a variety of methods such as

mixed models, Bayesian methods, and machine learning methods. Recent studies in different species have shown that gene expression levels can produce higher prediction accuracy than genotypes. However, only a few prediction methods were used in these studies. Thus, a comprehensive assessment of methods is needed to fully evaluate the potential of gene expression as a predictor of complex trait phenotypes.

Here, we used data from the *Drosophila* Genetic Reference Panel (DGRP) to compare the ability of several existing regression methods to predict starvation resistance from gene expression in the two sexes separately. The methods considered differ in the assumptions about the distribution of gene effect sizes – ranging from models that assume that every gene affects the trait to more sparse models – and their ability to capture gene-gene interactions. We also used functional annotation such as Gene Ontology (GO) as an external source of biological information to inform prediction models.

The results show that differences in prediction accuracy between methods exist, although they are generally not large. Machine learning methods tended to perform worse than the other methods, while Bayesian variable selection methods provided the highest prediction accuracy in both sexes. Incorporating GO annotations into Bayesian models further improved prediction accuracy and highlighted a few GO terms that were particularly predictive of the trait.

Our results confirmed the potential of transcriptomic prediction and highlighted the importance of selecting appropriate methods and strategies in order to achieve accurate predictions.

947T **Mediation analysis of key hepatic drug metabolizing enzymes and transporters in collaborative cross mice to characterize causal pathways of genetic regulation of drug metabolism as a resource for pharmacogenetics** Teresa McGee[1], Yanwei Cai[1], J. Scott Eaddy[2], John K Fallon[3], Rani J Qasem[3], Phil Smith[3], Merrie Mosedale[3], William Valdar[1] [1]Genetics, University of North Carolina at Chapel Hill, [2]Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, [3]Eshelman School of Pharmacy, University of North Carolina at Chapel Hill

Genetic variation in drug metabolism and disposition contributes to patient variability in drug response. Preclinical studies to test drug toxicity are often conducted in animal models that do not reflect human genetic diversity and fail to accurately predict human drug responses. The Collaborative Cross (CC) mouse genetic reference population is a promising preclinical model to more accurately model potential drug toxicity in humans. However, characterization of genes affecting drug response in CC mice has so far been limited. To investigate genetic effects on drug metabolism, we performed quantitative trait loci (QTL) mapping in 45 CC strains on both gene expression level and targeted proteomics measurements of key drug-metabolizing enzyme and transporter (DMET) genes. This revealed multiple genetic loci associated with variation in the DMET pathway. To study causal relationships between these loci, proteins, and transcripts, we used mediation analysis, testing for relationships between each pQTL, potential transcript mediators and protein levels. In particular, whereas standard mediation analysis tests relationships between a causal SNP (X), the phenotype of interest (Y), and a mediator (M), limiting the method to the analysis of a single mediator, we build upon previous methods to consider multiple mediators simultaneously. We extended our laboratory's existing Bayesian model selection framework for mediation analysis, bmediatR, to test multiple potential mediators for one proteomics phenotype. We apply this model to DMET data to better characterize causal pathways for variation in drug metabolism. This study provides a comprehensive analysis of DMET pathway variation in the CC population, and provides a rich data set to support the design and analysis of future pharmacogenetic studies in the CC.

948T **Chromosome 15 hotspot robustly associated with CAST/EiJ tuberculosis resistance** Rachel K Meade[1], Mohab Helmy[2], Marco Gontijo[1], Bailey Francis[2], Sherry Kurtz[3], Fred Boehm[4], Karen Elkins[3], Thomas Keane[2], Clare M Smith[1] [1]Molecular Genetics & Microbiology, Duke University, [2]European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Genome Campus, [3]Center for Biologics Evaluation and Research, Food and Drug Administration, [4]Biostatistics, University of Michigan

Each pathogenic infection is part of an evolutionary battle between host and invader. For *Mycobacterium tuberculosis* (*Mtb*), the causal agent of tuberculosis (TB), a host-pathogen arms race spans over millennia, making *Mtb* an enduring adversary of the human species. TB prognoses vary, from asymptomatic infection to severe morbidity, implicating host determinants of TB outcome. Mice are powerful, reproducible mammalian systems able to dissect complex infection dynamics, yet classical mouse studies often utilize only one host genotype, limiting understanding of diverse *Mtb* immunity. C57BL/6J (B6) mounts an IFN&gamma driven, T_H1-skewed response leading to relative TB resistance, robustly modeling only one TB outcome. To model natural human diversity, the Collaborative Cross (CC) mouse panel was born. This systems genetics resource consists of ~100 strains bred by intercrossing wild-derived and laboratory inbred lines; each CC strain has a unique yet reproducible host genome.

To illustrate the spectrum of *Mtb* immunity, we infected 50+ CC strains and measured disease metrics at a month post-infection. Comparing collected traits to the CC genomes, we mapped quantitative trait loci (QTL) associated with TB outcomes.

We located a 2Mb hotspot on Chromosome 15, containing 3 overlapping QTL, significantly linked with TB resistance. CC strains that inherited CAST/EiJ (CAST) haplotype at this locus exhibited lower lung *Mtb* burden and IL17 levels. To validate screen findings, we infected an F2 cohort bred from *Mtb*-resistant CC001, which inherited the Chr15 locus from CAST, and *Mtb*-susceptible CC042, which did not. F2 mice with the CAST region exhibited lower IFN&gamma levels in lung. This result replicates a unique feature of CAST mice, which exhibit very low lung IFN&gamma levels during infection, highlighting a non-canonical TB resistance mechanism.

To assess genetic features underlying the QTL hotspot, we used a novel telomere-to-telomere CAST genome assembly to reveal the unique chromosome architecture and strain-specific genes in the hotspot that may enable this unique *Mtb* response. We are also breeding congenic mice, which will allow mechanistic study of the region while refining the causal interval. Genetic and functional interrogation of this novel T_H1- and T_H17-independent TB resistance complex will expand field understanding of *Mtb* immunopathology. This investigation highlights genomic diversity as a vital host protective strategy against pathogens as prolific as *Mtb*.

949T **Mutational biases contribute to the neutral complexification of protein interaction networks following gene duplication** Angel F Cisneros Caballero[1], Lou Nielly-Thibault[1], Saurav Mallik[2], Emmanuel D Levy[2], Christian R Landry[1] [1]Université Laval, [2]Weizmann Institute of Science

Biological systems can gain complexity over time. At the molecular level, one example is heteromeric complexes replacing homomeric ones following gene duplication. While some of these transitions are likely driven by natural selection, we lack a neutral model to examine if such replacements could take place through the action of mutations alone. Here we build a biophysical model and examine if and how, without selection for new functions, mutations alone could favor these transitions. We use available protein structures to infer distributions of biophysical effects of mutations and simulate the evolution of homomers and heteromers following gene duplication. For most structures, the relative concentration of the heteromer increases over time, even when there is no inherent benefit of doing so. Different factors affect this trend, such as the distribution of mutational effects, the relative supply of mutations affecting synthesis rates, and differences in specific activity among the complexes. Our results show mutations alone can neutrally favor the accumulation of more complex protein quaternary structures, and that natural selection would be needed to reverse this tendency.

950T **Interpreting SNP heritability in admixed populations** Jinguo Huang[1], Saonli Basu[2], Mark D Shriver[1], Arslan A Zaidi[2] [1]Penn State University, [2]University of Minnesota

Heritability (h2) is central to our ability to describe, predict, and infer the genetic variation underlying complex traits. SNP heritability (h2SNP) allows one to estimate heritability in unrelated individuals and is an important aspect of the design of genetic studies. Despite the simple intuition and utility of h2SNP, its interpretation and equivalence to h2 is unclear, particularly in the presence of population structure and assortative mating, which limits our understanding of complex traits in admixed populations. It is known that h2SNP estimates can be inflated in structured populations because of confounding due to shared environment and linkage disequilibrium. Here, we present a theoretical analysis of the effect of population structure in admixed populations on h2SNP estimation.

We show that h2SNP estimates are biased in admixed populations compared to h2, even in the absence of confounding and even when all causal variants are known. We traced the source of this bias to two assumptions underlying random-effects models used to estimate h2SNP: (i) that the causal effects are independent, and (ii) that the population mates randomly. Both assumptions can be violated in recently admixed populations, leading to bias in h2SNP estimates. Importantly, h2SNP can be under- or over-estimated and we describe the behavior of the bias as a function of admixture history, ancestry-based assortative mating, and genetic architecture of the trait. We show that we can correct the bias in h2SNP estimates, but it is not always possible to do so, especially if individual ancestry is included as a covariate in the model – a common practice intended to correct for confounding effects of shared environment. This practice can result in overcorrection and will therefore yield underestimates of h2SNP, regardless of trait architecture.

Our findings provide a clear interpretation of SNP heritability in admixed and structured populations, where h2SNP estimate is not an unbiased estimator. We interpret the difference between h2 and h2SNP not as bias in the estimate, but because the estimand h2SNP itself as defined under the random-effects model is not equal to h2. We discuss the implications of the discrepancy between the h2SNP and h2 for the design of genome-wide association studies and polygenic risk prediction in diverse cohorts.

951T **A Litmus Test for Confounding in Polygenic Scores** Samuel Pattillo Smith[1], Hakhamanesh Mostafavi[2], Jeremy Berg[3], Dandan Peng[4], Graham Coop[5], Michael D Edge[4], Arbel Harpak[1] [1]Department of Population Health, University of Texas at Austin, [2]Department of Population Health, New York University, [3]Department of Human Genetics, University of

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Polygenic scores (PGS) are being rapidly adopted for trait prediction in the clinic and beyond. In addition to "direct" effects of one's genotype on one's trait value, PGS and their predictive accuracy can depend on environmentally or culturally-mediated factors such as Stratification, Assortative mating, and Dynastic (indirect) genetic effects ("SAD effects"). Despite the accumulating qualitative evidence, there is currently no estimation method to quantify the relative contribution of SAD effects to variation in a given PGS. We therefore developed a method that measures how much of the variance in a PGS (in a given sample) is driven by direct effects, SAD effects and their covariance. We leverage a comparison of a standard-GWAS based PGS and a PGS based on a sibling-GWAS—which is immune to SAD effects. Furthermore, the method breaks down effect variance components into principal axes of genetic ancestry, allowing for a nuanced interpretation of SAD effects. We applied our method to a range of PGS from the UK Biobank and large meta-analyses for anthropometric, biomedical and behavioral traits and found large and interpretable SAD signals in some of them. For example, we estimate that most of the variance in height PGS based on the GIANT study is appropriated to SAD effects and nearly 40% of the variance aligns specifically with the main North-South genetic ancestry axis, highly suggestive of stratification effects. Our method shows how the power of large GWAS can be married with the articulation of family designs to aid in the interpretation of PGS.

952T **F_{ST} based marker prioritization within QTL regions and its impact on genomic selection accuracy: Insights from a simulation study with high-density marker panels** Sajjad Toghiani[1], Samual Aggrey[2,3], Romdhane Rekaya[3,4] [1]USDA, [2]Department of Poultry Science, The University of Georgia, [3]Institute of Bioinformatics, The University of Georgia, [4]Department of Animal and Dairy Science, The University of Georgia

High-density SNP panels and sequence data have advanced the study of complex traits. However, the increase in the number of markers will likely result in a high rate of false positives and a potential reduction in genomic selection (GS) accuracy. The fixation index (F_{ST}) can be used to identify genome regions under selection pressure that can be prioritized in association analyses. In this study, the impact of different F_{ST} prioritization strategies on the estimation of the heritability and accuracy of GS were assessed. The QMSim software was used to generate datasets for a trait with heritability of 0.4 (SIM1) and 0.1 (SIM2). In both scenarios, the total genetic variance for the trait was assumed to be explained by 500 QTL. Each dataset was replicated 5 times. The simulated population comprised 30K animals from the last two generations of a ten-generation selective breeding program and genotyped using a 600K SNP marker panel. The correlation between true and estimated breeding values for 5,000 randomly selected animals from the last generation was used to assess the accuracy of GS. The top and bottom 5% of genotyped animals in the 9th generation, based on phenotype distribution, were grouped in two subpopulations and the F_{ST} scores were calculated for all 600K SNPs. The proposed algorithm consisted in computing the average F_{ST} scores for different SNP-windows (50, 100, 200, and 400 SNPs) surrounding each QTL position. SNP-windows with average F_{ST} scores surpassing a predefined threshold (e.g., 25% quantile of F_{ST} score distributions) were identified. Two F_{ST} prioritization scenarios were evaluated: 1) the 1% SNPs with the highest F_{ST} score were prioritized, and 2) Twelve SNPs within each SNP-window surrounding each QTL were prioritized based on their F_{ST} scores. Using all 600K SNPs, GBLUP-based prediction accuracy and estimated heritability for SIM1 (SIM2) were 0.77 (0.66) and 0.37 (0.10), respectively. However, prioritizing SNPs based on F_{ST} scores, particularly by selecting a 50 SNP-window around each QTL and randomly prioritizing 12 SNPs within that window resulted in a substantial increase GS accuracy of SIM1 (0.88) and SIM2 (0.78). Heritability estimates were slightly lower (0.34 for SIM1 and 0.09 for SIM2) compared to when all SNPs were used. The 50 SNP-window approach outperformed the 1% SNPs with the highest F_{ST} scores prioritization scenario. Testing of the algorithm when the number and position of QTL are unknown is warranted.

953T **Childhood sleep duration modifies the cumulative effects of fat mass-associated variants on BMI among preschool children** Mengna Zhang[1], Jianfei Lin[2], Shijian Liu[3], Fan Jiang[4], Hao Mei[5] [1]Data Science, University of Mississippi Medical Center, [2]Department of Developmental and Behavioral Pediatrics, Shanghai Jiao Tong University, [3]Department of Clinical Epidemiology and Biostatistics, Shanghai Jiao Tong University, [4]Shanghai Jiao Tong University, [5]Department of Data Science, University of Mississippi Medical Center

Background: Childhood obesity results from the combination of genetic and environmental factors, with sleep playing an increasingly significant role. Accumulating evidence has identified that sleep duration acts as a regulator of fat mass-associated genes in relation to obesity, but conflicting results were reported. This study aims to employ polygenic risk score (PRS) to confirm known obesity-related loci in preschool children and to investigate the impact of sleep duration on these associations in children.

Methods: 271 preschool children were recruited from the cross-sectional study from April to June 2011 in Shanghai, China. They all completed questionnaires, had physical exams, and provided blood samples. Ten genes linked to childhood obesity,

sleep issues, and HPA-axis regulation were genotyped. The cumulative effect of candidate variants for target traits were evaluated through unweighted polygenic risk score, while controlling for age, biological sex, and appetite. To study the modification effect of sleep duration, we created a PRS_{BMI} weighted from an external BMI GWAS and compared its effect on obesity and anthropometric traits between children with short and adequate sleep.

Results: After quality control, 21 SNPs remained. The unweighted PRS comprising these 21 variants showed a positive association with obesity (Beta=1.56; SE=0.43; p=0.00), and various anthropometric measurements. Using weights and a p-value threshold of 0.05 applies to a BMI GWAS in the Biobank Japan, three variants were selected to construct the PRS_{BMI}. Our cohort revealed that sleep duration modified the associations between the PRS_{BMI} and BMI, weight, arm circumference, and subscapular skinfold. In the group with adequate sleep, each unit increase in standardized PRS_{BMI} corresponded to increased BMI (Beta=0.28; SE=0.14; p=0.05), weight (Beta=0.57; SE=0.26; p=0.03), arm circumference (Beta=0.29; SE=0.14; p=0.04), and subscapular skinfold (Beta=0.32; SE=0.15; p=0.04), while such effects were absent in the short sleep group.

Conclusion: Candidate genetic variants cumulatively contribute to childhood obesity, BMI, and several anthropometric traits. Short sleep duration diminishes the genetic effects of selected fat mass and obesity-associated SNPs on BMI, weight, arm circumference, and subscapular skinfold among children. Our findings offer insights for informing future strategies to reduce childhood obesity by improving lifestyles, with a focus on sleep conditions.

954T **MAGE: Sources of gene expression in a globally diverse human cohort** Dylan J. Taylor[1], Surya B. Chhetri[2], Michael G. Tassia[1], Arjun Biddanda[1], Alexis Battle[2,3,4,5], Rajiv C. McCoy[1] [1]Department of Biology, Johns Hopkins University, [2]Department of Biomedical Engineering, Johns Hopkins University, [3]Department of Computer Science, Johns Hopkins University, [4]Department of Genetic Medicine, Johns Hopkins University, [5]Malone Center for Engineering in Healthcare, Johns Hopkins University

Genetic variation influencing gene expression and splicing is a key source of phenotypic diversity. Though invaluable, studies investigating these links in humans have been strongly biased toward participants of European ancestries, diminishing generalizability and hindering functional and evolutionary research. To address these limitations, we developed MAGE, a resource for **M**ulti-ancestry **A**nalysis of **G**ene **E**xpression. MAGE is an open-access RNA-seq data set of lymphoblastoid cell lines from 731 individuals from the 1000 Genomes Project spread across 5 continental groups and 26 populations. Most variation in gene expression (92%) and splicing (95%) was distributed within versus between populations, mirroring variation in DNA sequence. We mapped associations between genetic variants and expression and splicing of nearby genes (cis-eQTLs and cis-sQTLs, respective), identifying >15,000 putatively causal eQTLs and >16,000 putatively causal sQTLs that are highly enriched for relevant epigenomic signatures. These include 1310 eQTLs and 1657 sQTLs that are largely private to previously underrepresented populations. Our data further indicate that the magnitude and direction of causal eQTL effects are highly consistent across populations and that apparent “population-specific” effects observed in previous studies were largely driven by low resolution or additional independent eQTLs of the same genes that were not detected. Together, our study expands understanding of gene expression diversity across human populations and provides an inclusive resource for studying the evolution and function of human genomes.

955T **A Bayesian model selection framework for categorizing gene-by-treatment effects for molecular count phenotypes** Yuriko Harigaya, Nana Matoba, Brandon Le, Jordan Valone, Jason Stein, Michael Love, William Valdar University of North Carolina at Chapel Hill

Genetic effects on a phenotype of interest can differ, sometimes markedly, in response to an applied treatment. Such gene-by-treatment interactions (GxT) can be highly consequential in biomedicine and agriculture. An effective approach to identifying GxT signals and gaining insight into molecular mechanisms is mapping quantitative trait loci (QTL) of molecular count phenotypes, such as gene expression and chromatin accessibility, under multiple treatment conditions. Current practice, however, exhibits at least two limitations. First, a typical mapping analysis returns a list of feature-SNP pairs with significant GxT interactions but does not provide a principled approach to prioritizing GxT interaction of particular types. For example, treatment may have an impact on individuals of a certain genotype but not on others. In other cases, with similarly significant GxT interactions, treatment may affect all individuals but to different extents depending on their genotype. Formally assigning probabilities to these cases can facilitate the prioritization of response molecular QTLs for further investigation. A second potential limitation is the frequent assumption of linearity between the phenotype and the genotype after a variance-stabilizing transformation, such as the logarithm, which is routinely applied to molecular count phenotypes. This can lead to nontrivial model misspecification and inaccurate inference. Previous studies have shown that, consistent with the biologically reasonable assumption of common allelic additivity on molecular traits, such as expression, the linear relationship holds in the original count scale, but not in the transformed scale (PMID: 29021289, 29073327).

To address the first limitation, we have developed a downstream method for categorizing response molecular QTLs. Our method uses Bayesian model selection and assigns posterior probabilities to different types of GxT interactions for a given

feature-SNP pair. To address the second limitation, we have adapted nonlinear regression to account for the inherent relationship between the genotype and phenotype. After simulation analysis, we apply our method to response molecular QTLs previously identified in human primary neural progenitor cells from genetically diverse fetal donors with and without growth stimulation (PMID: 36798360). Our method provides an intuitive way to report the evidence for different types of GxT interaction across a set of feature-SNP pairs.

956T **Genomic regulation of pedicel characteristics of apple** Jairam Baba Danao[1], Jahed Khalil[2], Kranthi Varala[3], Peter Hirst[3] [1]Horticulture, Purdue University, [2]Alson H. Smith Jr. Agricultural Research and Extension Center, Virginia Tech, [3]Horticulture, Purdue university

Apple fruit development is significantly influenced by the pedicel, a slender stalk attaching the fruit to the tree. This study delves into the genetic control of pedicel characteristics, which are complex traits regulated by multiple genes. Our research focuses on two hybrid apple populations: 'Twenty Ounce' x 'Prairie Fire' crab apple and 'Edward VII' x 'Prairie Fire' crab apple. These populations vary in size, allowing us to investigate how pedicel attributes relate to apple fruit size. Our previous work established a clear connection between pedicel features and apple fruit mass. Specifically, pedicel length shows an inverse relationship, while pedicel diameter is directly related to fruit mass. Shorter, broader pedicels putatively facilitate improved water and nutrient transport. Efforts have been taken in *Arabidopsis thaliana* and *Nicotiana tabacum* to identify genes governing pedicel development. In *Arabidopsis thaliana*, overexpressing BREVIPEDICELLUS (BP) leads to excessive pedicel tissue growth, while loss of BP reduces its radius. When combined with a loss-of-function erecta (er), this reduction intensifies, causing radial constriction. In *Nicotiana tabacum*, knockdown of MADS-box gene NtSVP caused elongation in pedicels, while overexpression resulted in much shortened pedicels. These studies suggest the function of MADS-box transcription factors in plant pedicel development. In the current study we sought to identify and understand the genomic basis for control of apple pedicel length and diameter. Knowledge of Quantitative Trait Locus (QTLs) and genes that affect pedicel characteristics in apples have potential applications in apple breeding and fruit production. The identification and manipulation of these genes holds the promise of developing new apple varieties with improved pedicel traits i.e. ultimately fruit mass and enhanced fruit quality.

957T **The strength of weird ties: positive genetic interactions are impactful yet overlooked** Mengyi Sun Cold Spring Harbor Laboratory

Genetic interaction, the phenomenon that perturbing two genes simultaneously produces a phenotype deviated from the expectation given the effects of the individual perturbations, often reveals functional linkage among genes and pathways. Based on the sign of deviation, genetic interaction can be classified into positive or negative interactions. We hypothesize that, because positive interactions are more likely to involve genes from different pathways compared to negative interactions, they are more likely to generate impactful innovations. We verify our hypothesis by coupling large-scale genetic interaction data in yeast to the PubMed database, a prominent literature database in biology. Surprisingly, we find that despite their importance, positive interactions are at least 50% less likely to be studied compared to negative interactions. We provide evidence that this under-exploration can be partially explained by the bias inherent in human heuristic strategy in finding innovation. Finally, we demonstrate that interpretable machine-learning techniques can potentially alleviate the bias and inefficiency of human heuristic search. These results also provide insights into how to accelerate scientific research in general.

958T **To be or not to be an auxotroph - The curious case of MET15** Nelson Castilho Coelho[1], Sauin Parikh[1], Branden van Oss[1], Aaron Wacholder[1], Ivan Belashov[2], Sara Zdancenwicz[2], Manuel Michaca[2], Jiazhen Xu[2], Yun Kang[3], Nathan Ward[3], Sang Yoon[3], Katherine McCourt[1], Jake McKee[1], Trey Ideker[4], Andrew vanDemark[5], Gina DeNicola[3], Anne-Ruxandra Carvunis[1] [1]Computational and Systems Biology, University of Pittsburgh School of Medicine, [2]University of Pittsburgh, [3]Moffitt Cancer Center, [4]University of California, [5]University of PIttsburgh

Since its discovery in the 1970s, the homocysteine synthase Met15 (also known as Met17 and Met25) has been considered essential for inorganic sulfur assimilation in yeast. The *MET15* gene has served as auxotrophic marker for hundreds of experiments carried out in yeast, especially those using large collections like the yeast deletion collection, which have been of extreme impact in the foundation of eukaryote genetics and systems biology.

Here, we demonstrate through structural and evolutionary modeling, *in vitro* kinetic assays, and genetic complementation, that an alternative homocysteine synthase encoded by the previously uncharacterized gene YLL058W enables cells lacking Met15 to assimilate enough inorganic sulfur for survival and proliferation. We show that toxic accumulation of the gas hydrogen sulfide explains the failure for these cells to grow in patches or liquid cultures, unless provided with exogenous methionine or other organosulfurs. The addition of a hydrogen sulfide chelator to the culture media, and propagation as colony grids, allow cells without Met15 to assimilate inorganic sulfur and grow, and cells with Met15 to achieve even higher

yields.

Contrary to what has been assumed for decades, Met15 is not essential for inorganic sulfur assimilation in yeast. Instead, *MET15* is the first example of a yeast gene whose loss conditionally prevents growth in a manner that depends on local gas exchange.

959T **Quantifying gene by environment interactions in the activity of protein degradation pathways** Randi R. Avery, Mahlon A. Collins, Frank W. Albert Genetics, Cell Biology, and Development, University of Minnesota

Protein degradation by the ubiquitin-proteasome system (UPS) is an essential process that regulates protein abundance and removes misfolded and damaged proteins from cells. We recently showed that protein degradation is a genetically complex trait shaped by variation throughout the genome, often in a pathway-specific manner. UPS activity is highly environment-dependent, and gene-by-environment interactions (GxE) are pervasive in complex trait genetics. However, to what extent genetic influences on the UPS are modified by environmental factors is almost entirely unknown.
To comprehensively explore GxE in the genetics of the UPS, we mapped genetic influences on the activity of six UPS pathways in eight environments in the yeast *Saccharomyces cerevisiae*. We tested a standard growth condition and environments that cause large, well-defined changes in UPS activity, including nutrient deprivation, protein misfolding, and proteasome inhibition and activation. We used high-throughput reporters to measure UPS activity in millions of single cells. We mapped loci that contain variation affecting UPS activity using a statistically powerful method based on whole-genome sequencing of pools of phenotypically extreme recombinant progeny of a cross between two yeast strains.
We found 419 loci affecting UPS activity across the 48 pathway/environment combinations. In line with previous work, many loci affected UPS activity in a pathway-specific manner. To test for GxE, we examined 1) locus presence/absence and 2) changes in locus effect direction (*i.e.,* one allele increases UPS activity in one environment, but decreases it in another) across environments for each pathway. Of the average of 8 loci found across pathways in the standard medium, 88% were also found in at least one other environment. Of loci that were shared across environments, 17% had a change in effect direction. Thus, most genetic effects on UPS activity are consistent across environments, with modest amounts of GxE.
Some loci exhibited strong GxE. For example, 25% of the changes in effect direction across environments mapped to the *HAP1* gene. This locus showed the strongest change in effect direction of all loci detected. Here, the same allele increased UPS activity in nutrient deprivation but decreased UPS activity in other environments. This “hotspot” locus is known to affect the expression of thousands of genes in *trans*. Indeed, most (84%) loci with change in effect direction occurred within 25 kilobases of a known hotspot, which is significantly more than loci with consistent effect direction (67%; $p = 0.0014$). This suggests that *trans*-acting effects that modify cellular states are a source of GxE in the UPS. Our results reveal instances of strong GxE in UPS activity against a backdrop of remarkably stable effects across environments.

960T **Environment-specific hubs of genetic interaction modify the phenotypic effects of genetic perturbations** Ilan Goldstein, Joseph J Hale, Ian Ehrenreich Molecular and Computational Biology SectionDepartment of Biological Sciences, University of Southern California

Interactions between genetic perturbations and segregating loci can cause perturbations to show different phenotypic effects across genetically distinct individuals. In addition, interactions between a genetic perturbation and segregating loci may vary across environments. To study these complex interactions on a genome scale, we used combinatorial DNA barcode sequencing to measure the fitness effects of 8,013 CRISPRi perturbations targeting 1,721 distinct genes in 169 yeast cross progeny (or segregants) grown in two environments (respiratory and fermentable media). We identified ~600 genes whose perturbation show different effects across segregants in at least one environment. Only 271 (%45) of these genes were found to have background dependent effects in both environments. To understand the interactions giving rise to background dependent effects within and between environments, we mapped ~700 loci that modify the phenotypic effect of one or more perturbation. Six ‘hub’ loci were found to modify the effect of many genetic perturbations. Only one of these hub loci was found to modify a large number of phenotypic effects in both environments. These results suggest that an individual’s response to perturbations is shaped by a network of perturbation-locus interactions that cannot be measured by approaches that examine natural variation on its own or perturbations in singular genetic backgrounds. Furthermore, these networks of genetic interaction may differ dramatically between environments.

961T **A neural network based framework for systematically modeling genotype-phenotype relationships** David G Mets[1,1], Ryan York[2], Prachee Avasthi[2] [1]Genetics, Arcadia Science, [2]Arcadia Science

Modern methods for predicting phenotypes from genotypes often assume genetic influences on phenotypes combine additively and independently despite extensive documentation of gene-gene and gene-environment interactions. Here, we present an artificial neural network-based modeling framework for predicting phenotypes from genotypes (and vice versa) that captures non-linear relationships and can model many phenotypes simultaneously. In simulated and empirical data we find

accurate phenotypic predictions (5-20 mean average percentage error) based on genotypes. The accuracy of these predictions relies both on capturing gene-gene interactions and on simultaneously modeling multiple phenotypes suggesting that establishing genotype-phenotype maps that model the systematic relationships among genes, environments, and phenotypes can better capture the underlying biology thant mappings that treat the factors driving phenotypic variation as independent.

962F **Master regulators of biological systems in higher dimensions** Holger Eble[1], Michael Joswig[1], Lisa Lamberti[2], Will Ludington[3,4] [1]TU Berlin, [2]ETH Zurich, [3]Carnegie Institution for Science, [4]Johns Hopkins University

A longstanding goal of biology is to identify the key genes and species that critically impact evolution, ecology, and health. Network analysis has revealed keystone species that regulate ecosystems and master regulators that regulate cellular genetic networks. Yet these studies have focused on pairwise biological interactions, which can be affected by the context of genetic background and other species present, generating higher-order interactions. The important regulators of higher-order interactions are unstudied. To address this, we applied a new high-dimensional geometry approach that quantifies epistasis in a fitness landscape to ask how individual genes and species influence the interactions in the rest of the biological network. We then generated and also reanalyzed 5-dimensional datasets (two genetic, two microbiome). We identified key genes (e.g. the rbs locus and pykF) and species (e.g. Lactobacilli) that control the interactions of many other genes and species. These higher-order master regulators can induce or suppress evolutionary and ecological diversification by controlling the topography of the fitness landscape. Thus, we provide a new method and mathematical justification for exploration of biological networks in higher dimensions.

963F **Stable genotype-to-phenotype mapping in an evolving population** Jessica Rhodes[1], Mark Bitter[2], Skyler Berardi[3], Jack Beltz[3], Hayes Oken[3], Lauren McIntyre[4], Dmitri Petrov[2], Paul Schmidt[3] [1]Genetics, Stanford University, [2]Genetics, Stanford, [3]Biology, University of Pennsylvania, [4]Molecular Genetics & Microbiology, University of Florida

Genome-wide association studies are widely used to infer the genetic basis of a range of complex traits across species. Yet, it is unknown how stable the resulting genotype-phenotype-map is throughout the evolutionary process. Specifically, polygenic changes in allele frequency may shift the epistatic landscape and increase or decrease the association of variants with the phenotype of interest. In this study, we leverage a highly replicated, outdoor mesocosm system of *D. melanogaster* populations to directly test the stability of the genotype-phenotype map throughout evolution. We mapped abdominal pigmentation – a classical, well-studied trait, with a known core biochemical network and associated variants from several previous GWAS studies. Our goal is to assess both how the genotype-to-phenotype map shifts while the population undergoes a seasonal shift in pigmentation and how well it predicts the underlying seasonal allele frequency changes.

The trait mapping of the outbred populations from both outdoor and indoor cages (N=10 for each treatment) at two timepoints revealed that the genotype-to-phenotype map was broadly conserved across replicate cages and timepoints. As expected, the average abdominal pigmentation decreased from spring to fall in outdoor cages. While outdoor populations exhibited much greater phenotypic shifts than indoor populations, the large effect size loci were conserved between them with a few notable exceptions – most prominently the *yellow* gene peak that only appears in the outdoor populations. Furthermore, our trait mapping is consistent with the largest hits of previous GWAS in other populations and we identified SNPs near the key pigmentation genes *tan*, *ebony*, and *bab*. However, when we tested whether these loci exhibit systematic shifts in allele frequencies associated with the overall pigmentation evolution from spring to fall, we found shifts at only one of the large-effect size loci identified during mapping (*bab*). We hypothesize that the muted impact of most mapped genes on the evolution of the trait may be driven by their pleiotropic effects. Overall, we find that the genotype-to-phenotype map is remarkably stable and similar between populations, but is not necessarily a robust predictor of which variants will be responsible for the phenotypic responses to selection.

964F **Let's talk about bruno: Uncovering genetic variants causative of differences in dysgenic sterility in Drosophila melanogaster.** Lorissa Full Saiz, Natalie Copeland, Julia Gorman, Emilio Espinosa, Oscar Full Olbera, Hasan Nooruddin, An Bui, Caycel Desales, Sydni Marsh, Bryan Salinas, Erin S Kelleher Biology and Biochemistry, University of Houston

Transposable elements (TEs) are genetic parasites that proliferate in the germlines of organisms to ensure their transmission to offspring. While TEs are predominantly vertically transmitted from parent to offspring, they can invade new host genomes via horizontal transfer between species. The unrestricted transposition of newly invaded TEs can reduce the fertility and fitness of their host. Although TE repression mechanisms evolve rapidly, it is not instantaneous, so standing variation in host populations with respect to TE fertility and fitness effects are predicted to be major targets of selection following TE invasions.

The P-element invasion of Drosophila melanogaster is a modern example of a novel TE invasion and provides us with

an opportunity to identify standing variation in the fitness effects of TEs. In the absence of host repression, P-elements cause germline DNA damage that leads to a sterility syndrome called hybrid dysgenesis. Contemporary populations of D. melanogaster evolved piRNA-mediated silencing of P-elements, in which maternally transmitted piRNAs suppress transposition and dysgenic sterility in offspring. However, P-element naive mothers derived from pre-invasion natural populations do not produce P-element piRNAs and are not able to transmit them to offspring. These offspring are then typically sterile. Therefore, differences in the frequency of dysgenic sterility between offspring of different naive female genotypes reveal the presence of host genetic variation in fertility costs.

Through quantitative trait locus (QTL) mapping, our lab identified the bruno locus as a likely source of natural variation in dysgenic sterility. However, the causative variant behind these fertility differences remains unknown. I am performing fine-scale, marker-assisted mapping to narrow the QTL window, then determining the exact causative variant(s) of the phenotypic differences in dysgenic sterility by performing CRISPR-Cas9 mediated allele swaps. Identifying the causative variants will allow us to determine how dysgenic sterility is reduced, and ultimately if this reduction was beneficial following P-element invasion. Although evolutionary biologists often localize natural variation through QTL mapping, it is rare to isolate the exact causative variant that determines a trait of interest. My research will complete the progression from QTL to causative variation, thereby expanding our understanding of the molecular genetic basis for trait variation.

965F **Genetic correlations associated with body coloration, aggression, and activity levels in *Drosophila melanogaster*** Paulina Montes Mendez, Samuel Miller, Katelyn McCaffery, Sarah Ruckman, Kimberly Hughes Florida State University

One long standing question in evolutionary biology is whether single genes that control multiple traits (pleiotropy) results in limitations on adaptive evolution. If it does, then our ability to predict adaptation (e.g., in the face of changing environments) is compromised. We are using a much-discussed correlation between body coloration and aggressive behavior as a system to address this question. Having found the predicted genetic correlation between cuticle color and aggressive behavior in *Drosophila melanogaster* using artificial selection (unpublished data), we then asked if any other behaviors co-evolved under selection on cuticle color. For example, the dopamine synthesis pathway is plausibly related to color, aggression, and other behaviors, such as activity level. We therefore measured activity level of flies (*D. melanogaster*) selected for darker and lighter cuticle color. Results suggest that activity level is higher in the dark-selected lines, suggesting that genetic correlations do constrain the independent evolution of cuticle color and behavior. The next step in this study to identify candidate genes underlying the correlation and test for pleiotropic effects using transgenic approaches.

966F **Genetic and morphological contributions to natural variation in pup isolation calls in deer mice** Maya L Woolfolk[1,2,3], Nicholas Jourjine[1,2,3], Sade McFadden[1,2,3], Hopi E Hoekstra[1,2,3] [1]Organismic and Evolutionary Biology, Harvard University, [2]Molecular and Cellular Biology, Harvard University, [3]Museum of Comparative Zoology, Harvard University

Neonatal vocalization is a behavior critical for eliciting care from parents across vertebrates and in many mammals, vocalizations also serve to establish social bonds between parents and offspring. While neonatal vocalization varies between species in both quantity and content, the proximate mechanisms underlying this variation are largely unknown. Deer mice (genus *Peromyscus*) are a group of closely related but behaviorally diverse rodents that offer an opportunity to study natural variation in pup vocal behavior. Previously, we identified significant interspecific differences in temporal and acoustic features of pup cries, several of which evolved repeatedly between species. To further investigate the genetic basis of this behavioral variation, we performed quantitative trait locus (QTL) mapping with 881 F2 individuals generated by intercrossing two sister species: *P. maniculatus* and *P. polionotus*. We first found acoustic features of different vocalization types are uncoupled in F2 hybrids, suggesting that they are under separate genetic control, and we identified several loci significantly associated with species-specific differences in pup vocal behavior. One likely cause underlying the acoustic variation between species is different laryngeal morphology. Therefore, in conjunction with our genetic approach, we also targeted the mechanistic basis of vocal variation by utilizing microCT-generated data and performing morphometric analyses of three cartilages in the primary vocal organ, the larynx. We found that *P. maniculatus* has larger thyroid cartilages and greater total larynx cartilage volume than *P. polionotus*. Both features are hypothesized to play an important role in acoustic structure and vocal production, which suggests there are potential contributions of differential laryngeal morphology to interspecific variation in acoustic features such as average frequency/pitch. We are following up this initial work by analyzing these laryngeal features in F2 hybrids, with which we can identify a causal relationship between structural variation and variation in acoustic structure. In this system, we are uncovering mechanisms underlying natural variation in pup vocal behavior to identify genetic and morphological bases of behavioral evolution.

967F **Haplotype reconstruction using low-pass whole-genome sequencing in genetically diverse mouse populations** Samuel J Widmayer[1], Lydia K Wooldridge[2], Michael C Saul[2], Laura Reinholdt[2], Beth L Dumont[2,2], Daniel M Gatti[2] [1]Computational Sciences, The Jackson Laboratory, [2]The Jackson Laboratory

The Diversity Outbred mouse population (DO) is a premier resource for powerful quantitative trait locus mapping and systems genetics. However, access and genotyping cost remain a barrier to entry for DO experiments. DO mice are typically genotyped using the Giga Mouse Universal Genotyping Array (GigaMUGA), which contains roughly 140,000 markers and costs $100 per mouse. GigaMUGA data demonstrate an estimated 12.2 crossovers per generation have accumulated between generations 21 and 36, which is roughly half the expected rate. This disparity suggests that genotyping DO animals using GigaMUGA arrays lacks the required resolution to capture all recombination events, reducing its utility for genetic mapping. We describe an accurate, cost-effective workflow for genotyping DO animals using low-pass whole-genome sequencing. We prepared 96 samples using two different sequencing approaches: double-digest restriction site–associated DNA sequencing (ddRADseq) and low-coverage whole genome sequencing (lcWGS). These samples were split between two populations: 48 DO animals (Generation 41) and 48 animals derived from an advanced intercross between four wild-derived strains (4WC) of *Mus musculus musculus* and *M. m. castaneus*. We constructed reference haplotypes for each population and used QUILT to impute SNPs for each sample. We then derived chromosome-specific genotype and allele probabilities from a filtered subset of these imputed SNPs using R/qtl2. Founder allele probabilities were on average over 95% concordant with GigaMUGA-derived allele probabilities in both populations for either library preparation method. We achieved comparable concordance even after downsampling the alignments to lower than 0.25X coverage, significantly reducing the cost of genotyping compared to the GigaMUGA. The observed number of crossovers in DO mice also closely approximates their expected frequency, suggesting that our workflow may improve the precision of haplotype reconstruction in complex mouse crosses. Hundreds of loci less than 0.1 Mb remain discordant from GigaMUGA, and ongoing work seeks to leverage recently published structural variant datasets and regions of identity-by-descent to characterize sources of noise in haplotype reconstructions. We aim to lower barriers to conducting DO mouse experiments by reducing genotyping costs and the computational expertise associated with obtaining accurate haplotype reconstructions.

968F **Trophic-level gut length divergence evolved under sexual conflict in Lake Malawi cichlids** Aldo Carmona Baez[1], Patrick J Ciccotto[2], Emily C Moore[3], Kaitlin P Coyle[4], Melissa S Lamm[5], Erin N Peterson[4], Natalie B Roberts[1], Rafael Guerrero[1], Reade Roberts[1] [1]Biological Sciences, North Carolina State University, [2]Warren Wilson College, [3]University of Denver, [4]Q2 Solutions, [5]Novogene

Variation in gastrointestinal morphology is associated with dietary specialization across the animal kingdom. Gut length generally correlates with trophic level, and increased gut length in herbivores is a classic example of adaptation to diets with lower nutrient content and a higher proportion of refractory material. However, the genetic basis of gut length variation remains understudied, partly due to the inaccessibility and plasticity of the gut tissue, as well as the lack of dietary diversity within traditional model organisms relative to that observed among species belonging to different trophic levels. Here, we confirm the genetic basis of gut length variation among recently evolved Lake Malawi cichlid fish species with various dietary adaptations. We then produce interspecific, inter-trophic-level hybrids to map evolved differences in intestinal length in an F2 mapping cross between *Aulonocara koningsi*, a carnivore with a relatively short gut, and *Metriaclima mbenjii*, an omnivore with a relatively long gut. We identify numerous candidate quantitative trait loci for evolved differences in intestinal length. These quantitative trait loci are predominantly sex-specific, suggesting an evolutionary history of sexual conflicts for the gut. We also identify epistatic interactions potentially associated with canalization and the maintenance of cryptic variation in the cichlid adaptive radiation. Overall, our results suggest complex, polygenic evolution of gut length variation related to trophic level differences among cichlids, including sexual conflicts and epistatic interactions that may be involved in evolutionary processes underlying other traits in cichlids.

969F **Toward whole-organism genetic mapping: The Arcadia Chlamydomonas Diversity Collection** Ryan York, Prachee Avasthi, Ben Braverman, Tara Essock-Burns, Galo Garcia, Jase Gehring, David Matus, Cameron MacQuarrie, David Mets Arcadia Science

How genomes give rise to organismal variation is a central problem in biology. Genetic diversity panels are powerful tools for this task, allowing diverse phenotypic measurements to be mapped to a common genomic background. In theory, measurements of a given diversity panel could be scaled to a point approximating the breadth of functions encoded by the genome, creating the potential for whole-organism genetic mapping. With this in mind, we created the Arcadia *Chlamydomonas* Diversity Collection (ACDC), a community resource for organism-level genotype-phenotype mapping. The ACDC consists of ~1,800 fully-sequenced, clonal F_2 strains generated from a cross between the unicellular algal species *C. reinhardtii* and *C. smithii. C. reinhardtii* and *C. smithii* display extensive morphological, behavioral, ecological, and physiological variation. Using high-throughput phenotyping tools, we have found that these differences are also reflected among the ACDC strains. Leveraging this, we have developed neural network-based genetic mapping approaches to jointly identify the molecular basis of many phenotypes at once. We have identified several principal axes of variation and their genetic correlates, providing an initial glimpse into the genomic underpinnings of *Chlamydomonas* organismal biology. We are excited to keep expanding the characterization of the ACDC and, as such, all strains, genotypes, phenotypes, and experimental tools

associated are publicly available and open source.

970F **Error rates in Q_{ST} vs. F_{ST} comparisons depend on genetic architecture** Junjian «Janis» Liu, Michael «Doc» Edge Department of Quantitative and Computational Biology, University of Southern California

Genetic and phenotypic variation among populations is one of the fundamental subjects of evolutionary genetics. One question that arises often in data on natural populations is whether differentiation on a particular trait might be caused in part by natural selection. For the past thirty years, researchers have used Q_{ST}-F_{ST} approaches to compare the amount of trait differentiation among populations on one or more traits (measured by the statistic Q_{ST}) with differentiation on genome-wide genetic variants (measured by F_{ST}). Theory says that under neutrality, F_{ST} and Q_{ST} should be approximately equal in expectation, so Q_{ST} much larger than FST is consistent with local adaptation, and Q_{ST} much smaller than F_{ST} is consistent with stabilizing selection. At the same time, in making this and similar statements, investigators have differed in their definitions of genome-wide F_{ST} (particularly regarding “ratio of averages” vs. “average of ratios” versions of F_{ST}) and in their definitions of the variance components in Q_{ST}. Here, we show that these details matter. The different versions of F_{ST} and Q_{ST} have different interpretations in terms of coalescence time, and comparing incompatible statistics can lead to elevated type I or type II error rates, with some typical choices leading to type I error rates as high as 15% when the nominal rate is 5%. Further, although the expectation of Q_{ST} does not depend on the number of loci influencing the trait, the distribution of Q_{ST} does, so the right choice of comparison distribution depends on genetic architecture. We discuss solutions and provide a guide to making Q_{ST}-F_{ST} comparisons that are theoretically coherent and empirically conservative.

971F **Like mother, like daughter? Phenotypic plasticity, environmental covariation, and heritability of size in a parthenogenetic wasp** Scott Monahan, Arun Sethuraman Biology, San Diego State University

Dinocampus coccinellae (Hymenoptera:Braconidae, Euphorinae) is a solitary, generalist Braconid parasitoid wasp that reproduces through thelytokous parthenogenesis, an asexual process in which diploid daughters emerge from unfertilized eggs, and uses over fifty diverse species of coccinellid ladybeetles worldwide as hosts. Here we utilized a common garden and reciprocal transplant experiment using parthenogenetic lines of D. coccinellae presented with three different host ladybeetle species of varying sizes, across multiple generations to investigate heritability, plasticity, and environmental covariation of body size. We expected positively correlated parent-offspring parasitoid regressions, indicative of heritable size variation, from unilineal (parent and offspring reared on same host species) lines, since these restrict environmental variation in phenotypes. In contrast, because multilineal (parent and offspring reared on different host species) lines would induce phenotypic plasticity of clones reared in varying environments, we expected negatively correlated parent-offspring parasitoid regressions. Contrary to expectations, our results indicate (1) little heritable variation in body size, (2) strong independence of offspring size on the host environment, (3) a consistent signal of size-host tradeoff wherein small mothers produced larger offspring, and vice versa, independent of host environment. Our study offers support for a constrained fecundity advantage model of Cope’s Law, wherein D. coccinellae maintains phenotypic plasticity in body size despite parthenogenetic reproduction.

972F **Co-mutation based genetic networks to infer temporal mutation dynamics in ancient human mitochondrial genomes** Rahul K Verma[1], Janna Fierst[2] [1]Biological Sciences, Florida International University, [2]Florida International University

The evolution of modern humans is a long and complicated process which started from their first appearance and continues to the present day. Various habitats have played a significant role in shaping the migratory paths in ancient times and stabilization of current sub-populations around the globe. Mitochondria is the chief cell organelle for responding to these habitats since it is involved in cellular metabolism and energy generation. The majority of human ancient DNA (aDNA) studies were focused mainly on mitochondrial DNA (mtDNA) due to the fact that mtDNA is present in cells in a higher copy number than the nuclear genome Studies have shown that mitochondrial DNA (mt-DNA) has acquired certain adaptations in response to human diet as well. To account for the role of genetic background with respect to variable sites in shaping the migratory and evolutionary path, the ancient mtDNA was analyzed as spatiotemporal co-mutation networks.

The human sub-populations are represented by mitochondrial haplogroups due to enrichment of variable sites geographically. Similarly, studies on ancient mtDNA revealed the dominance of haplogroup U in hunter-gatherer class and haplogroup H along with J and K in early farmer class of ancient humans. We employed a network-based approach to deduce the co-occurring variable sites to generate the weighted genetic interaction networks for the mtDNA sequences for six eras named Mesolithic, Neolithic, Copper, Bronze, Iron and Middle era. We found that Mesolithic era had highest percentage (6.8%) and the Middle era had least percentage (1.0%) of ancestral SNPs, which is consistent with the period of that era.

The haplogroup composition showed that, Mesolithic era is completely dominated by haplogroup U, in Neolithic and Copper eras haplogroups U, H and K are dominant. Haplogroup U has been associated with hunter-gatherer group and haplogroup H has been associated with farmer group. In Copper, Iron and Middle eras haplogroup H was found to be dominantly present

which supports the idea of agricultural developments during these periods.

The analysis of genetic interaction networks provided evidence for the significance of different genetic interactions in each era. In Mesolithic era ND5 with ND4 and CYB with ND4 genes, in Neolithic era, ND1 gene with CO3 and CYB genes and ND5 gene with multiple genes, in the Copper era CO1 gene with ND6 and ND2 with ND5, in Bronze era, ND4 with ND1 and ND6 with ND5 and CO1 with multiple genes, and in Iron era, ND6 gene with ND3, CYB and ND5, and CYB, ND4, ND1 and ND2 genes forming prominently high weight interactions.

Since the networks are purely temporal, we further sought to perform the phylogenetic studies on these mtDNA sequences to generate the spatio-temporal evolutionary relation among the sequences. This would help us to compare the network based temporal results with the geography-based phylogeny.

973F **The gall of an aphid : Novel secreted proteins hijack plant gene expression** Aishwarya Korgaonkar, David Stern, Clair Han HHMI Janelia Research Campus

In an extreme form of interspecies exploitation, insects hijack plant developmental pathways to induce novel plant organs called galls. These galls represent a dramatic reprogramming of plant cell biology resulting from the direct action of insects on host plants. One example are the cone-shaped galls induced by the aphid, *Hormaphis cornu,* on the leaves of *Hamamelis virginiana*.

We discovered that these aphids encode a large class (~500) of novel secreted, cysteine-rich proteins with no homology to any known proteins. These proteins are highly divergent other than a pair of widely spaced cysteine-tyrosine-cysteine (CYC) motifs, hence named *bicycle* proteins. Evidence for these proteins as gall effectors comes from our discovery that links a *bicycle* gene, *g16073*, to a naturally occurring variation in plant gall color. Furthermore, the aphid genotype at this genetic locus has a targeted and quantifiable effect on plant gene expression leading to the hypothesis that the transformation of leaf tissue into a gall is driven by the direct action of injected aphid bicycle proteins. Ongoing work is focused on functional characterization of aphid bicycle proteins in the host plant.

974F **Leveraging old data and new methods to illustrate the critical role of epistasis in genetics and evolution** Jorja Elliott[1], Maximos Chin[2], Brian E Fontenot[3], Sabyasachi Mandal[1], Thomas McKnight[1], Jeffery P Demuth[3], Heath Blackmon[1] [1]Texas A&M University, [2]University of California Davis, [3]University of Texas at Arlington

Much of evolutionary theory is predicated on assumptions about the relative importance of simple additive versus complex epistatic genetic architectures. Previous work suggests traits strongly associated with fitness will lack additive genetic variation, whereas traits less strongly associated with fitness are expected to exhibit more additive genetic variation. We use a quantitative genetics method, line cross analysis, to infer genetic architectures that contribute to trait divergence. By parsing over 1600 datasets by trait type, clade, and cross divergence, we estimated the relative importance of epistasis across the tree of life. In our comparison between life history traits and morphological traits, we found greater epistatic contributions to life history traits. Our comparison between plants and animals showed that animals have more epistatic contribution to trait divergence than plants. In our comparison of within-species versus between-species crosses, we found that only animals exhibit a greater epistatic contribution to trait divergence as divergence increases. While many scientists have argued that epistasis is ultimately of little importance, our results show that epistasis underlies much of trait divergence and must be accounted for in theory and practical applications like domestication, conservation breeding design, and understanding complex diseases.

975F **Coheritability and Coenvironmentability as Concepts for Partitioning the Phenotypic Correlation** Jorge Vasquez-Kool Health Human and Life Sciences, Shaw University

Central to the study of joint inheritance of quantitative traits is the determination of the degree of association between two phenotypic characters, and to quantify the relative contribution of shared genetic and environmental components influencing such relationship. One way to approach this problem builds on classical quantitative genetics theory, where the phenotypic correlation between two traits is modelled as the sum of a genetic component called the coheritability, which reflects the degree of shared genetics influences affecting the phenotypic correlation, and an environmental component, namely the coenvironmentability that accounts for all other factors affecting the observed trait-trait association. Here a mathematical and statistical framework is presented on this partition and describe visualization tools to analyze concurrently the coheritability, coenvironmentability and the phenotypic correlation. Examples of its application are presented.

976F **Exploiting natural variation in wild *Saccharomyces cerevisiae* strains to understand why certain individuals are more susceptible to alpha-synuclein (a-syn) toxicity** Julio A Molina Pineda[1], Manasa Veluvolu[2], Tara N Stuecker[2], Stephan N Witt[3],

Jeffrey A Lewis[2] [1]Cell and Molecular Biology, University of Arkansas, [2]Biological Sciences, University of Arkansas, [3]Biochemistry and Molecular Biology, LSU Health Shreveport

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting more than 5 million people worldwide. A hallmark of PD is dopaminergic neuronal cell death caused by cytotoxic aggregates of alpha-synuclein (a-syn). However, it is unclear why a-syn forms cytotoxic aggregates in some individuals but not others, and how these a-syn aggregates cause cellular toxicity. Understanding the genetic basis of variation in a-syn cytotoxicity in humans is challenging, so we turned to the budding yeast Saccharomyces *cerevisiae* as a powerful and tractable genetic model. a-Syn overexpression in yeast recapitulates the subcellular localization and cytotoxicity seen in neuronal cells, and, excitingly, we have identified wild yeast strains with either increased susceptibility or high resistance to a-syn overexpression. Thus, we have a new model for understanding the genetic basis of natural variation in a-syn susceptibility. Using a panel of strains with high or low a-syn susceptibility, we have performed global transcriptional profiling following over-expression of a-syn using two different promoter systems. Our results suggest that strains with natural resistance to a-syn over-expression have a more robust gene expression response, while also identifying candidate genes that protect against a-syn toxicity or exacerbate it. Overall, this work demonstrates the power of using natural variation combined with system genetics to identify novel genes and processes important for disease susceptibility.

977F **QTL Mapping of Yeast Mating Efficiency and Cell Cycle Progression** Dominick Costanzo[1], Dimitra Aggeli[1], Alex Nguyen Ba[2], Michael Desai[3], Gregory Lang[1] [1]Lehigh University, [2]University of Toronto, [3]Harvard University

Most phenotypes are complex, with contributions from many loci throughout the genome. Quantitative Trait Locus (QTL) mapping is a powerful strategy for uncovering the loci contributing to complex traits, including small-effect variants. We are using a mapping population of ~100,000 segregants from a cross between a laboratory strain and a vineyard isolate. Each segregant has been genotyped and uniquely barcoded. Using a bulk barcoded assay, we mapped loci underlying two traits: mating efficiency and cell- cycle progression. We are currently applying this technique to plasmid copy number and signaling through the mating pathway.

978F **Dissecting complex traits in yeast by saturation genome editing** Kevin R Roy[1,2], Justin D Smith[1], Shengdi Li[3], Sibylle C Vonesch[3], Michelle Nguyen[1], Wallace T Burnett[1], Kevin M Orsley[1], James E Haber[4], Robert P St.Onge[5], Lars M Steinmetz[1,2,3] [1]Genetics, Stanford University, [2]Stanford Genome Technology Center, Stanford University School of Medicine, [3]Genome Biology Unit, European Molecular Biology Laboratory, [4]Rosenstiel Basic Medical Sciences Research Center and Department of Biology, Brandeis University, [5]Biochemistry, Stanford University

Understanding how genetic variants give rise to complex traits remains one of the biggest challenges in genetics. We previously developed a high-throughput CRISPR guide/donor homology-directed repair (HDR)-based system for variant phenotyping termed ***Multiplexed Accurate Genome Editing with Short, Trackable, Integrated Cellular barcodes*** (MAGESTIC). To characterize the efficiency and fidelity of MAGESTIC at the genome scale, we introduced guide-donor libraries targeting natural single-nucleotide variants (SNVs) into the laboratory strain and characterized on- and off-target editing outcomes by whole-genome sequencing of thousands of clones. This work revealed four major challenges: (1) low efficiency guides leading to unedited clones, (2) repetitive regions prone to structural variant (SV) formation, (3) integration of donor plasmids at target sites, and (4) low representation of SNVs due to re-cleavage by SpCas9. To address the first two challenges, we developed a super-charged donor HDR system (MAGESTIC 3.0) combining three orthogonal methods: donor DNA recruitment with the FHA domain of yeast Fkh1p, single-stranded donor DNA synthesis with the bacterial retron system, and *in vivo* assembly of linearized donor plasmids. MAGESTIC 3.0 improved editing kinetics at low-efficiency targets and editing fidelity at SV-prone regions over each single system, completely abrogating aberrant SV formation in some cases. To address the third challenge, we developed an inducible SaCas9 system to cleave integrated plasmids and simultaneously recover correct edits with near 100% efficiency. To address the fourth challenge, we profiled a panel of engineered variants of SpCas9 and LbCas12a with enhanced fidelity and broadened target ranges for their ability to edit all SNVs across guide target regions. We identified several variants showing substantially improved SNV representation over wild-type SpCas9. We used this optimized MAGESTIC 3.0 system to screen all targetable natural variants residing in 112 quantitative trait loci (QTL) for 32 conditions, revealing a complex genotype-phenotype map of causal variants. As expected, causal variants were more likely to be missense variants. Surprisingly, a large fraction of QTL harbored more than one causal variant with implications for association-based studies. MAGESTIC 3.0 will enable the functional dissection of the genome at single-nucleotide resolution and inform attempts to improve HDR-based genome editing across organisms.

979F **Selection to combined antifungal drug stressors produces non-additive genomic responses in experimentally-evolved *Saccharomyces cerevisiae* populations** Megan A Sandoval-Powers, Molly K Burke Integrative Biology, Oregon State University

The role of additive versus non-additive genetic interactions on complex trait variation remains an open question in quantitative genetics. These interactions are often highly influenced by environmental perturbations; therefore, a deeper understanding of how quantitative trait loci respond to complex stressful environments is needed. Here, we used an evolve-and-resequence (E&R) framework to study the genomic and phenotypic responses associated with antifungal drug resistance, a human health-relevant complex trait. Outbred *Saccharomyces cerevisiae* populations were evolved under a single antifungal drug environment (caspofungin acetate [Cas] or clotrimazole [Clo]) or a combined drug environment (Cas+Clo) for ~50 asexual generations. By comparing changes in populations evolved to the combined drug treatment with the single drug treatments, we assessed the extent to which responses were driven by additive effects (predicted by individual stressors) versus non-additive effects (unique from individual stressors). Phenotypic changes in evolved populations suggest that the combined-drug populations increased the greatest in fitness relative to the ancestral population. Pooled-population genome sequencing of evolved replicates revealed ~40K high-coverage single-nucleotide polymorphisms (SNPs) to investigate within and between treatments. We used linear models to identify candidate SNPs, those with the greatest and most consistent change in frequency over time, and little overlap was observed across treatments when comparing single-drug and combined-drug populations. Candidate SNPs often implicated different genes and regions, most notably when compared between Cas and Cas+Clo treatments. A significantly differentiated SNP in the Cas treatment occurred within chromosome 15 in the *PDR5* gene encoding a pleiotropic drug resistance factor, and SNPs in this gene were not significant in the Clo or Cas+Clo treatments. Distinct genomic changes in the combined drug-treatment indicate that polymorphisms can contribute non-additively to the drug resistance trait. This finding suggests that genetic interactions may be more complex than predicted by simple additivity, at least in the context of antimicrobial drug resistance. This has important implications for our understanding of how variation arises and evolves in complex traits associated with human health and disease.

980S **Mapping the Genetic Underpinnings of Natural Variation in Mutagen Tolerance in *Drosophila melanogaster*** Llewellyn Green, Erin Kelleher Biology and Biochemistry, The University of Houston

Organisms are continuously under attack by numerous mutagens in the environment. If this damage is not repaired correctly, it can result in increased mutation and genomic instability. Apart from these obvious direct effects on DNA damage and mutation, many of these mutagenic agents can also exert other harmful physiological effects on the organism, including death. Consequently, mutagen tolerance, the ability to withstand and promptly repair DNA damage, is a fundamental determinant of organismal fitness.

Genetic screens in individual strains provide limited biological insight into how natural populations respond to mutagen exposure. Despite its relative importance, little is known about the genetic factors that contribute to natural variation in mutagen tolerance. To address this gap, we used the Drosophila Synthetic Population Resource (DSPR) to perform an extreme quantitative trait locus (QTL) mapping strategy to map mutagen tolerance in *Drosophila melanogaster.*

We chose to focus on two common environmental mutagens, ionizing radiation (X-rays) and UV-B radiation. These mutagens differ significantly in their variance and prevalence in the environment. Higher-energy ionizing radiation predominantly causes double-stranded breaks (DSBs), while UV-B radiation causes more variable types of damage that may involve separate repair pathways. We predict that due to these differences in exposure, there will be a wider range of genetic variation for UV-B tolerance to act upon than for ionizing radiation.

Our third instar screen on ionising radiation identified a single locus that spans the third centromere, while the UV-B screen on embryos identified three loci that have not been previously associated with the trait. The lack of overlap between the two stressors suggests that different DNA repair and stress response pathways are acting in each case. While a small number of genes familiar to DSB repair have been identified under the ionising radiation QTL, very few familiar candidates have been found under the mapped UV-B loci; a potential indication that the genes contributing to tolerance in natural populations of *D. melanogaster* are much broader than previously characterised.

We anticipate that with the addition of functional genetic analysis of candidate genes under mutagen tolerance QTLs, coupled with SV analysis of founder haplotypes, will be used to identify the genetic underpinnings of natural variation in mutagen tolerance in *D. melanogaster* and provide new insights into the evolution of stress response mechanisms.

981S **The genetic basis of the non-monotonic response to atrazine in *Drosophila melanogaster*** Pamela C Lovejoy Biology, St. Joseph›s University New York

Atrazine is one of the most widely used herbicides in the United States. Even though atrazine has agricultural benefits, it has been shown to have a variety of negative effects on non-target organisms. In *Drosophila melanogaster*, atrazine causes reductions in lifespan, alterations to development time, decreases in reproductive fitness, and changes in expression of

proteins involved in response to oxidative stress. Within several of these traits, non-monotonic dose response curves have been observed, such that intermediate concentrations of atrazine have the strongest effects, while low and high concentrations of atrazine have minimal effects. In this study, I investigated the effect of atrazine on the trait of egg laying preference. Flies given the choice of five different atrazine concentrations lay the most eggs on food containing the highest and lowest concentrations and the least eggs on food containing intermediate concentrations. This creates a non-monotonic "U-shaped" curve when number of eggs laid is graphed. The goal of this research is to identify and investigate the genes affecting this non-monotonic response. To do this, I have performed a genome-wide association study (GWAS) using the *Drosophila* Genetic Reference Panel (DGRP). The GWAS returned 138 genetic variants that are associated with the non-monotonic response to atrazine. The candidate genes associated with these variants are currently being investigated with RNAi. This research was integrated into the BIO 290 Modern Genetics course at St. Joseph's University New York (SJNY) over several years to provide the students with a course-based undergraduate research experience (CURE) during their sophomore year. Students assisted with data collection for the complete GWAS and performed their own smaller GWAS with the subset of data they collected during the course, identifying candidate genes and common gene ontology terms. Students presented posters with their results at the SJNY Undergraduate Research Symposium, gaining a valuable experience that will enhance their CVs going forward. This research will help elucidate the non-monotonic effects of atrazine and increase college student retention and future success through its integration into the classroom.

982S **Evolutionary Constraints Associated with Color and Aggression in *Drosophila melanogaster* and *D. simulans*** Sarah Ruckman, Kimberly Hughes Florida State University

One long standing question in evolutionary biology is whether single genes that control multiple traits (pleiotropy) result in limitations on adaptive evolution. If it does, then our ability to predict adaptation (e.g., in the face of changing environments) is compromised. We are using a much-discussed correlation between body coloration and behavior as a system to address this question. Aggression and pigmentation are hypothesized to be genetically correlated in many organisms because the same biochemical pathways (e.g., dopamine) are used in color synthesis and the production of molecules that modulate behavior, including aggression. We used two species of fruit flies, *Drosophila melanogaster* and *D. simulans* to test this hypothesis. By focusing on two species, we assessed the repeatability and similarity in genes underlying genetic correlations. To test for genetic correlation, we first selected for darker (and lighter) body color and then tested for aggressive behavior periodically. Our results suggest that dark-selected flies evolved to be more aggressive, suggesting that genetic correlation does constrain the independent evolution of cuticle color and behavior. The next step in this study to identify candidate genes underlying the correlation and test for pleiotropic effects using transgenic approaches.

983S **Genetic dissection of variation in heavy metal susceptibility in *Drosophila melanogaster*** Stuart Macdonald Molecular Biosciences, University of Kansas

Environmental toxicants present considerable risk to human health, and among the most concerning are compounds containing heavy metals. Due to broad industrial use and historically broad incorporation into common products, there is widespread metal contamination of drinking water, food, and soil. Even extremely low levels of exposure to certain metals can yield deleterious consequences for human health. However, there is also considerable variation in an individual's response to any given toxic compound. Understanding the genetic and molecular pathways underlying the response to toxic metals, and the nature of differential metal susceptibility, will enable more accurate prediction of the risks associated with exposure. Detailed, population-based studies in humans are challenged by the inability to control the dose to which individuals are exposed. Genetically-tractable model systems such as *Drosophila* offer considerable advantages; Exposure levels can be precisely controlled, powerful genetic mapping experiments can be executed, and candidate susceptibility genes can be functionally validated using a sophisticated genetics toolkit. Additionally, there is broad gene conservation between humans and flies, including many known metal response and detoxification genes. Thus, studies in flies can provide fundamental insight into toxicity variation in humans. We exposed adult flies from >500 different inbred strains from the *Drosophila* Synthetic Population Resource (DSPR) to toxic levels of 5 metal salts (cadmium chloride, lead acetate, lead nitrate, manganese chloride, and mercury chloride). The DSPR is a powerful multi-parental population framework enabling the genetic dissection of trait variation. QTL mapping led to the identification of QTL for all metals, some of which positionally overlapped across treatments, implying they have common genetic effects on susceptibility to different metals. This is supported by positive correlations in strain lifespan across all exposure treatments, strong correlations in the founder haplotype effects at coincident QTL, and the recapitulation of 4 multi-metal loci following QTL analysis of a composite phenotype resulting from principal components analysis of all metal response traits. Mapped QTL implicate several plausible candidate genes that we are functionally testing via RNAi and genome editing. For instance, *GstS1* (encoding a glutathione S transferase detoxification protein), *Gss1* and *Gss2* (glutathione synthases that are also involved in xenobiotic detoxification), and *ZnT41F* (a zinc transporter). In combination with ongoing expression QTL studies in DSPR strains under both naive and metal-exposed conditions, our work will lead to the identification of loci and mechanisms contributing to variation in a series

of key environmental and industrial metal toxicants.

984S **Increasing power in inbred strain association mapping by recognizing variance heterogeneity** Marissa C Ashner[1], Teresa M McGee[2], Robert W Corty[2], Jialiu Xie[1], William Valdar[3] [1]Department of Biostatistics, University of North Carolina at Chapel Hill, [2]Curriculum in Bioinformatics and Computational Biology, University of North Carolina at Chapel Hill, [3]Department of Genetics, University of North Carolina at Chapel Hill

Modern quantitative trait locus (QTL) mapping in panels of inbred strains uses a linear mixed model (LMM) to test for SNP-phenotype association while accounting for a random effect of population structure. A decade of mathematical tricks has mitigated the computational expense of repeatedly fitting this complex model for genome-wide applications. Existing procedures, however, assume that the phenotype of each strain (or individual) is known with equal precision. In reality, this assumption often does not hold: individuals from some strains are intrinsically more variable than others, and ignoring this can lead to false positives and missed QTL signals. We propose a method, weighted Inbred Strain Association Mapping (wISAM), that accounts for heteroscedastic residual variance in the study population using a weighted regression technique and makes use of variance shrinkage methods to stably estimate these weights. We compare wISAM to existing methods through simulation, demonstrating that it can provide additional statistical power for GWAS, and in real data applications on using the Hybrid Mouse Diversity Panel (HMDP) and panels of inbred strains in other organisms. We also consider how to accommodate potentially problematic features of some datasets, including phenotypic distributions with zero-inflation or experimental designs where some strains have only a single biological replicate.

985S **Exploring evolutionary forces shaping the genetic architecture of complex disease** Kellen Riall[1], Jeremy Berg[2] [1]he Department of Human Genetics, The University of Chicago, [2]The Department of Human Genetics, The University of Chicago

Genome-wide association studies (GWAS) have shed light on the genetic basis of complex diseases, but the role of evolutionary forces in shaping genetic variation of disease phenotypes remains unclear. Despite the presumed fitness cost of disease and strong evidence for selection against functional mutations, evidence for sustained directional selection against complex disease phenotypes is limited. Motivated by this puzzle, we propose a model for a highly polygenic disease evolving at mutation-selection-drift balance (MSDB). Our model assumes that complex disease variants are subject to a combination of directional selection against a disease phenotype and stabilizing selection on related pleiotropic quantitative traits, and predicts that our ability to learn about directional selection against disease from GWAS data depends on our assumptions about the source of mutational pressure. If the mutational pressure conforms to long-term evolutionary equilibrium, where mutations predominantly increase disease risk, directional selection is easily detectable, but the predicted patterns of variation are inconsistent with the data. In contrast, if mutations are equally likely to raise or lower disease risk, the ability to detect directional selection depends on the strength of stabilizing selection. When stabilizing selection is weak relative to directional selection, the allele frequency distribution of risk increasing alleles is indistinguishable from that obtained under neutrality, and contains no information about the strength of directional selection against on the disease. However, this symmetry can be broken if we know whether the risk increasing alleles are derived or ancestral. In contrast, when stabilizing selection is strong, this relationship is reversed: the derived versus ancestral identity of risk increasing alleles is relatively uninformative, but directional selection creates an excess of risk increasing alleles at high frequencies relative to low frequencies. Our analysis suggests that a systematic characterization of the impact of sustained directional selection on disease phenotypes will require that we can determine whether individual risk increasing causal variants are derived or ancestral. To determine if this is feasible, we study the concordance of derived versus ancestral status among putative causal variants within fine mapping credible sets for several UK Biobank traits.

986S **Contemporary evolution of complex traits in the plant fungal pathogen Zymoseptoria tritici** Camille Maurin[1], Marc-Henri Lebrun[2], Cyrille Saintenac[1], Anne Genissel[1] [1]INRAE, [2]CNRS

How pathogens evolve new virulence remains a important question for Health and Agriculture. Microbes, especially fungi, have a high potential for rapid adaptation, with excessive levels of genetic diversity. Here we study the ascomycete fungus *Zymoseptoria tritici*, the causal agent of Septoria Tritici Blotch, a major disease of wheat across the world.

Beyond doubt the arms race between host and pathogen is shaping the fungal genome, evolving against the host immune system. However the overall view on the genetic basis of rapid adaptation of fungal populations remains unclear. A recent resistance breakdown in the field against the resistance gene Stb16q thus provides a unique opportunity to study contemporary evolution of ecologically relevant phenotypes. Towards this goal we sampled a panel of 109 natural fungal isolates. To seek loci conveying adaptation to host resistance, we carried out a thorough study combining phenotyping in the laboratory, NGS sequencing of 109 natural isolates, together with pacbio sequencing of several virulent isolates.

Here we provide empirical evidence using GWAS and QTL mapping approaches that several loci are involved, with polymorphism arising from standing genetic variation or *de novo* mutations. The relative contribution of both types of variants is discussed in our study by comparing empirical data to forward-in-time simulations. Candidate loci are currently under functional survey, by producing gene knockout and allele replacement. In addition, we found that both coding and regulatory variants contribute to the phenotypic variation. To further investigate the role of non-coding *cis*-regulatory mutations and explore the nature of gene regulatory networks involved in this adaptation, we collected samples during infection of wheat isolines in presence or absence of the resistance gene in the laboratory. Gene expression measurements using 3'sequencing is currently ongoing.

Altogether, our results demonstrates the high complexity of this ecologically relevant and recent adaptation still ongoing in the field.

987S **The effect of Neanderthal introgression on human complex traits** Leqi Tian, David Lu, April Wei Cornell University

Early modern humans interbred with Neanderthals after migrating out of Africa approximately 50,000 years ago, resulting in around 1-4% of the genomes of present-day non-African descendants being derived from Neanderthals. Recent studies have shown that the remaining introgressed variants in present-day humans generally exhibit reduced phenotypic effects and per-SNP trait heritability compared to variants originated in humans, but the evolutionary models to explain the reduction remain elusive. Previous evolutionary studies of Neanderthal introgression have focused on purifying selection against deleterious mutations by modeling direct fitness costs; this approach does not provide much insight into how Neanderthal introgression influences human complex traits. Here, we examine Neanderthal introgression through the lens of complex trait evolution to gain a more comprehensive understanding of this process. We implement a forward-in-time simulation model that is specifically designed for the evolutionary history of introgression and complex traits evolution which incorporates a human exon annotation map from the UCSC Genome Browser and the deCODE recombination map. Tree-sequence format is used to encode the whole-genome data during simulations, which allows us to quantify the impact of introgression on the phenotypes of modern humans as well as the distribution of Neanderthal ancestry across the entire genome accurately and efficiently. A vast parameter space encompassing polygenicity, the distribution of effect sizes, and modes of selection on phenotypes is simulated to investigate more realistic models for explaining the observed data. We are also testing whether stabilizing or directional selection could better explain the distribution of Neanderthal ancestry in human genomes and the reduced influence of the remaining introgressed variants on human complex traits. These results will provide new insights into the impact of Neanderthal introgression on human complex traits evolution.

988S **A novel method for computing population summary statistics** Ziqing Pan[1], Drew DeHaas[2], April Wei[2] [1]Computational Biology, Cornell University, [2]Cornell University

Population level summary statistics, such as allele frequency, linkage disequilibrium and SNP association effect, are essential in comprehending the population history, natural selection, and disease mechanisms. Methods for computing population-level summary statistics are predominately implemented on genotype matrices, which do not take advantage of the correlation among samples or SNPs. Furthermore, the computation complexity via matrix algebra can sometimes increase quadratically with sample size or number of SNPs. As a result, existing computational frameworks often fail to afford large-scale complicated computations with large datasets. Here, we propose a novel alternative method for computing whole-genome summary statistics by leveraging a new data structure genotype representation graph (GRG) developed in our group. Like variant call format (VCF) and ancestral recombination graph (ARG), GRG can encode whole-genome polymorphism data losslessly, but it is more compact. The radical reduction in size comes from capturing the shared sample list across mutations with shared paths in the graph such that each node represents a unique subset of samples that share mutations. Given the graph representation of genomic data, we reformulate the traditional population summary statistics computation (e.g., matrix-matrix multiplication) as arithmetic along graph traversals. Leveraging the hierarchical structure, we further reduce the computational complexity by reusing the computed nodes to avoid repeated calculation. Several simple first-order summary statistics including allele frequency and association effect have been computed for the whole-genome with the novel algorithm, achieving the same order of time as the current state-of-the-art methods. Moreover, we anticipate graph traversal on GRG will promise better scalability for more complex and higher order statistics that are currently unsolvable with the traditional methods, such as genome-wide linkage disequilibrium. The GRG-based computation will open up new avenues for efficient and scalable genomic computation in the era of large biobanks.

989S **Testing for differences in polygenic scores in the presence of confounding** Jennifer Blanc, Jeremy J Berg University of Chicago

Polygenic scores have become an important tool in human evolutionary genetics, enabling the prediction of individual phenotypes from their genotypes. Understanding how the pattern of differences in polygenic score predictions across

individuals intersects with variation in ancestry can provide insights into the evolutionary forces acting on the trait in question. However, because most polygenic scores are computed using effect estimates from population samples, they are susceptible to confounding by both genetic and environmental effects that are correlated with ancestry. The extent to which this confounding drives patterns in the distribution of polygenic scores depends on patterns of population structure in both the original estimation panel and in the prediction panel. Here, we use theory from population and statistical genetics, together with simulations, to study the procedure of testing for an association between polygenic scores and axes of ancestry variation in the presence of confounding. We use a general model of genetic relatedness to describe how confounding in the estimation panel biases the distribution of polygenic scores in a way that depends on the degree of overlap in population structure between panels. Using the understanding gained from this analysis, we develop a method that leverages the patterns of genetic similarity between the two panels to guard against these biases, and show that this method can provide better protection against confounding than the standard PCA-based approach in certain situations. We then use the UK Biobank to further test our approach and explore how various parameters, such as sample size and number of loci, impact both PCA and our procedure. Finally, we apply our approach to re-analyze several cases of reported polygenic selection in humans utilizing state-of-art GWAS and polygenic score construction methods that maximize the predictive power of the polygenic score while controlling for stratification bias.

990S **QTL-mapping using the ancestral recombination graph** Vivian Link, Charleston W. K. Chiang, Nicholas Mancuso, Michael D. Edge University of Southern California

Understanding the genetic basis of complex phenotypes is a central pursuit of human medical genetics. Our recent method finds associations between phenotypes and genetic loci by using the Ancestral Recombination Graph (ARG). The ARG encodes the evolutionary history of all individuals in a sample, and can be seen as a series of marginal coalescent trees. Conditioned on the ARG, we calculate local expectations of the Genetic Relatedness Matrix (eGRM), which we test for association in a variance components framework using Restricted Maximization Likelihood (REML).

Our simulation results show that the eGRM efficiently captures untyped variation and that our method is especially beneficial for phenotypes with multiple causal alleles in the same locus (allelic heterogeneity). However, our method does not scale well to sample sizes that are larger than a few thousand individuals. Here, we present strategies for making our method faster, including approximating the eGRM and approximating the REML algorithm. We further assess how the approximations affect our power to find associations.

991S **Muskox genetics: How does genotype affect complex wool fiber phenotypes?** Dominique N Wagner[1,2], Victor N Roman[1,2], Evon Delisle[1], Clayton N Allex-Buckner[1,2], Amber N Braddock[1,2], Sanaz N Farajollahi[1,2], Patrick B Dennis[1], Blake W Stamps[1], Heather J Huson[3], Nancy Kelley-Loughnane[1] [1]Biomaterials Branch, Air Force Research Laboratory, [2]Biological and Nanoscale Materials, Eqlipse Technologies, [3]College of Agriculture and Life Sciences, Cornell University

Understanding the link between genotype and phenotype is critical for the understanding of any trait. However, this link becomes increasingly difficult to discern with complex traits, which often involve dozens or hundreds of genes. Muskox (*Ovibos moschatus*) wool, known as qiviut, is hypothesized to have greater thermal insulative properties than other natural or synthetic fibers. However, due to the relatively low number of commercial producers and low individual fiber yield (usually <4kg/year), availability of qiviut is extremely limited. As such, there is significant interest in understanding the genetic basis for fiber quality and yield, with the eventual goal of increasing production and integrating fiber assembly into a synthetic biological framework. To understand the link between genotype and the complex qiviut fiber phenotype and materials chemistry of muskox keratin proteins, we generated a reference genome and performed whole genome sequencing on 20 individuals collected from The Musk Ox Farm (Palmer, AK) with a range of wool fiber quality and production quantity. We performed a genome-wide association study to identify single nucleotide polymorphisms (SNPs) that are significantly associated with both fiber quality and yield. To address the likelihood that multiple genes/SNPs contribute to these complex phenotypes, we also performed a random forest analysis that identified which ensemble set of genomic features is most likely to contribute to the phenotypes of interest. These analyses will help us understand which genomic features are likely to be important to qiviut fiber quality and production, and will advance our ability to integrate the portions of the biological fiber production mechanism into a synthetic framework.

992S **Experimental evolution of mating system in M. guttatus** Sharif Tusuubira, John Kelly KU

We apply experimental evolution to Mimulus guttatus (yellow monkeyflower) to replicate populations in three different treatments: fully outcrossing, mixed mating, and fully selfing. Replicate populations were allowed to evolve in response to selection pressures imposed by their respective mating system treatments for 10 generations. We then grew seed from the ancestral population alongside the final generation of each experimental population to measure trait divergence. We show

that allele frequency changes at QTLs and changes in overall homozygosity (an immediate affect of mating system differences) are both responsible for differing trait means among populations. Distinguishing these causes, we find that numerous traits responded to selection to increase the efficiency of self-fertilization whenever it was a major component of reproductive success. Whole genome sequencing applied to the evolved populations reveals a signature of selection on individual loci as well as the broad effects of mating system differences on molecular evolution via linked selection.

993S **The genetic architecture of adherence in a clinical isolate of the budding yeast *Saccharomyces cerevisiae*** Hita Yalla[1], Atari Abundo[1], Brendan Woodworth[1], Helen Murphy[2] [1]Biology, William & Mary, [2]William and Mary

Biofilms are communities of microbes that have enhanced resistance to environmental stressors. They are characterized by an extracellular matrix, attachment to a surface, and cell-to-cell adherence. Biofilms can pose a threat in medical settings due to the challenges of eradicating them. Some *Saccharomyces cerevisiae* strains isolated from natural and clinical environments are able to form biofilms when induced in nutrient limiting conditions. We investigated the genetic architecture of adherence to a plastic surface, the first step in biofilm formation, in a highly heterozygous clinical isolate that contained ~40,000 SNPs when originally isolated. The fluorescent protein mCherry was incorporated into the genome, which allowed for a high-throughput fluorescence-based assay to measure adherence in an F_5 mapping population. Using bulk segregant analysis (BSA-seq) on pools of highly adherent and non-adherent segregants, we found 5 major loci that contribute to variation in plastic adherence. Of particular interest was natural allelic variation in *TPK2*, one of the catalytic subunits of PKA. Allele replacement in a highly-adherent segregant verified the role of a single SNP contributing significant variation to plastic adherence. These findings suggest that allelic variation that effects cAMP-PKA signaling is a major source of variation in plastic adherence, as it is for other filamentous phenotypes, such as invasive growth and biofilm colony formation in yeast. Our results also suggest that environmental isolates harbor genetic variation in major signaling pathways that can contribute to pathogenic and virulence traits.

994S **(co)Evolution of GAPDH genes following duplication in yeast** Mohammad Siddiq[1,1], Nick Brown[2], Hannah Kania[3], Patricia Wittkopp[4] [1]Ecology and Evolutionary Biology & Molecular, Cellular, and Developmental Biology, University of Michigan, [2]University of Pennsylvania, [3]Duke University, [4]Department of Ecology and Evolutionary Biology, University of Michigan

Evolutionarily related copies of genes formed from historical duplication events, called paralogs, are present in almost all known genomes. Paralogs are initially redundant in function following gene duplication, and this redundancy enables them to evolve and functionally diversify in ways that were previously inaccessible. Despite this diversification, proteins encoded by paralogous genes often retain the capacity to carry out some common ancestral functions. Whether this functional overlap plays a role in the subsequent evolution of paralogs is not well understood. We investigated how three paralogous genes that encode *GAPDH*s in *Saccharomyces cerevisiae—TDH1*, *TDH2*, and *TDH3*—diversified during historical evolution and evaluated whether these genes exert coevolutionary pressures on each other in the present day. We found that the paralogous *TDH* genes have evolved to contribute differently to growth, and that this divergence is largely explained by evolved differences in the genes' cis-regulatory sequences. Further, our results suggest that the conserved ancestral capacity of all *TDH* genes to provide *GAPDH* activity continues to intertwine their evolutionary fates such that the evolution of variation at one gene is contingent on the level of activity being provided by others. The propensity of evolutionarily related genes to retain functional overlap and coevolve may enable the net output of a metabolic step to be maintained by stabilizing selection while the relative contributions of the underlying genes change. This phenomenon, akin to observations in developmental pathways, may allow the architecture of metabolic steps to drift over time and provide a simple explanation for why similar metabolic reactions are carried out in different ways across evolution.

995S **Investigating the Evolution of Duplicated Genes Using Conditional Complex Genetic Interaction Analysis** Brittany M. Greco, Rohan Dandage, Soyeon Cho, Elena Kuzmin Biology, Concordia University

Whole-genome duplication (WGD) events have played a pivotal role in shaping the genomes of organisms across the tree of life. *Saccharomyces cerevisiae* underwent WGD approximately 100 million years ago which corresponds to the time of the emergence of angiosperms (and their fruit) and has since retained around 18% of its paralogs. The factors influencing the retention and functional evolution of specific duplicates remain poorly understood. In this study, we use a systematic analysis of conditional complex genetic interactions to unravel the impact of metabolic changes in the environment on the retention of paralogs resulting from the WGD event. We quantitatively scored the fitness of 79 double and corresponding 158 single gene deletion mutants of WGD paralogs, which have previously shown to exhibit sparse digenic and trigenic interaction profiles under standard growth conditions, across a range of metabolic environmental conditions comprising nine carbon and five nitrogen sources for a total of 45 carbon-nitrogen combinations. We have also considered alternative metabolic conditions that simulate the emergence of fruity plants which have been shown to illicit a phenotypic response in our

paralogs of interests. Conditions which led to a growth defect of the most mutant strains contained monosodium glutamate combined with either raffinose or sucrose, proline with ribose, urea with glucose and allantoin with fructose, while urea with ribose affected the least. Each paralog pair showed a growth defect in at least one condition. By mapping conditional trigenic interactions of WGD paralogs, we aim to measure their degree of functional divergence and redundancy and reveal their functional roles. This study will provide insight into the evolutionary forces that shape genomes.

996S **Fine-mapping causal polymorphisms underlying differential protein expression in *Saccharomyces cerevisiae*** Minh Phan[1], Maggie Barry[2], Winter A Yi[1], Daniel Pollard[1] [1]Biology, Western Washington University, [2]University of Oregon

The central dogma outlines the flow of genetic information from DNA to mRNA to protein, underscoring the influence of genetic variation on observable traits in organisms. Genetic variants can exert their effect on observable phenotypes by changing the function, localization, and importantly, abundance of mRNA and protein. Previous research largely focused on mRNA abundance and used it as a proxy for protein abundance. However, there are processes independent of mRNA abundance like protein synthesis and degradation can have significant effects on protein abundance. Thus, how genetic variation affects protein abundance and the molecular mechanisms underlying this process presents itself as an under-explored yet crucial aspect in the story of gene expression.

To answer this question, a dynamic system will be modeled with the eukaryotic organism *Saccharomyces cerevisiae* (baker's yeast) and its easily inducible mating pheromone response pathway. To decode the genetic basis of trait variation, a key step is to identify the genetic variants associated with the phenotypic trait. While the current bulk segregant analysis (BSA) method efficiently maps causal quantitative trait loci (QTLs), there has yet to be a method to fine-map the specific single nucleotide polymorphism (SNP) within QTLs responsible for phenotypic traits. Our project aims to address this gap with a novel fine-mapping method that integrates BSA with a recombination selection scheme. Iterative selections for recombinants with specific marker combinations will ensure that genetic recombination has occurred between each SNP, overcoming the resolution limitation of the classic BSA QTL mapping method and facilitating the identification of individual causal SNPs. This fine-mapping method will be developed using the case study of a two-gene, CAM1 and DIG1, locus that has been previously shown to be a trans-acting QTL affecting the protein expression of the pheromone-responsive gene, FIG1. An efficient, high-throughput fine-mapping method will further bridge the gap between genetic variation and protein expression and potentially uncover molecular mechanisms underlying this process.

997T ***egg-7,* an ortholog of human phosphoglucomutase 3 is required for fertility in *C. elegans*** Katherine Maniates[1], Kendall Flanagan[2], Andrew Singson[2] [1]Rutgers University, [2]Waksman Institute, Rutgers University

Fertilization and the activation of gametes are precise events that require cell-cell adhesion, binding, fusion, signaling, and recognition. Despite the importance of fertilization for all sexually reproducing organisms, many of the genes and molecules involved in generating the fertilization synapse are still unknown. In particular, many fewer genes and molecules on the egg have been identified as being involved in fertilization. The asymmetry between known sperm and egg components required for fertilization is seen across many organisms including mice, flies, worms, and fish. Identification of these genes required in the egg for fertilization has been challenging due to both biological and technical reasons, among these reasons are redundancy, pleiotropy, and the complexity of these processes. Many of these challenges are not unique to identifying genes involved in fertilization, but also in any field that encounters sterile or lethal mutant phenotypes.

We have developed a forward genetic screening approach using recently developed balancer chromosomes to identify new genes required for fertilization. The benefits of this screen include generating stable balanced lines of candidate mutations that are sterile or lethal following EMS Mutagenesis for further analysis. This approach will be valuable for researchers across fields that are searching for sterile or lethal mutants. Preliminary rounds of this screen have identified many candidate mutations. One mutant that we identified is a premature stop codon in F21D5.1, now called *egg-7,* an ortholog of mammalian phosphoglucomutase 3 (PGM3). PGM3 is an enzyme involved in glycosylation and synthesis of GPI anchors. Mutants in this gene are egg-specific sterile, hermaphrodite animals have normal morphology and germline development. However, these mutants are sterile, and fertility is unable to be recovered after mating with males. EGG-7 is localized in the oogenic germline and early embryos. We hypothesize that *egg-7* is required for glycosylation and synthesis of GPI anchor proteins on the oocyte's surface for fertilization.

998T **Investigating the role of optimal apoptosis levels in maintaining progeny fitness and fertility under temperature stress** Kristen Quaglia, Hannah N Lorenzen, Samantha H Oswald, Lisa N Petrella Marquette University

As surface temperatures rise due to global warming, organisms from rice to flies to nematodes face extinction solely due to the inherent temperature sensitivity of fertility. We investigated how germline apoptosis may be a mechanism that can preserve fertility under temperature stress. In the pioneer species *C. elegans*, we use both genetically diverse wildtype strains

and mutants that affect the level of apoptosis to investigate this question. Under non-stress conditions, approximately 50% of oogenic nuclei in the germline undergo apoptosis to remove low-quality nuclei and/or to supply more cytoplasm to remaining oocytes in the N2 wildtype strain. It has been shown that as temperature increases and fertility decreases, apoptosis in the germline increases over baseline levels. To understand how progeny fitness and fertility may be impacted by apoptosis, we are investigating how the level of apoptosis correlates with fertility and progeny fitness measured by oocyte size and embryonic lethality. We found that genetically diverse wildtype strains showed a range in levels of apoptosis, fertility, and progeny fitness metrics at elevated temperature, but that the rate of apoptosis did not directly correlate with the level of fertility or progeny fitness. However, all strains were able to increase apoptosis during temperature stress, suggesting that it may be the ability to change apoptosis levels that is important. Therefore, we tested fertility and progeny fitness traits in mutants that had no apoptosis or elevated levels of apoptosis under non-stress conditions. We found that mutants with no apoptosis had lower fertility, smaller oocytes, and higher embryo lethality at all temperatures compared to the N2 wild type. We also found that mutants with high apoptosis had lower fertility, smaller oocytes, and higher embryo lethality than N2 wild type at higher temperatures, but the same as N2 wild type at non-stress temperatures. Our results indicated that there may be an optimal range of apoptosis in the *C. elegans* germline. Both no apoptosis, and high apoptosis lower fertility and progeny fitness at elevated temperatures compared to most wild strains. Apoptosis within an optimal range may function under a moderate temperature stress to maintain fertility and ensure fit progeny.

999T **The role of SPE-21, a palmitoyltransferase in sperm activation.** Saai Anugraha Tiruchendurai Suryanarayanan, Amber Krauchunas, Dawn Chen, Andrew Singson Rutgers, The State University of New Jersey

Spermatids undergo post-meiotic differentiation to gain polarity, become motile and fertilization competent. In *C. elegans*, this process, also known as sperm activation, transforms round spermatids to motile, amoeboidal spermatozoa. These spermatozoa have pseudopods that help them crawl to the oocytes. Additionally, during sperm activation, Golgi-derived vesicles called membranous organelles (MOs) fuse with sperm plasma membrane and release their contents to the membrane and extracellularly. This process is analogous to mammalian acrosomal reaction. Using forward genetics, we identified a mutation in s*pe-21* also designated as *dhhc-5*, *spe-21(as41ts)*. We also generated a genetic null, *dhhc-5(syb4299)*, using CRISPR. *spe-21* is on chromosome III, and it encodes a palmitoyl acyltransferase (PATs). This is only the second identified mutation of the 15 predicted PAT encoding genes in *C. elegans*. We observed the following phenotypes in the *spe-21* mutant worms: Both *spe-21* mutant hermaphrodites and males are sterile. Their sperm fail to activate *in-vitro* and *in-vivo* and do not produce pseudopods. SPE-21 localizes to the MOs. Using split-ubiquitin membrane yeast -2-hybrid, we have data suggesting potential interactions of SPE-21 with other proteins involved in spermatogenesis, sperm activation and fertilization. Our discovery will be pivotal in underscoring the importance of palmitoylating enzymes in making fertilization competent sperm in *C. elegans* and other species.

1000T **SPE-13 is a sperm membrane protein involved in the fertilization synapse complex function during fertilization in C. elegans** Yamei Zuo[1], Amber Krauchunas[2], Andrew Singson[1] [1]Rutgers University, [2]University of Delaware

Fertilization is a process where two gametes recognize and fuse to form a zygote that would later develop into an embryo. However, the underlying genetic mechanisms for fertilization are still not well understood in all species. spe-13 has been identified as a “spe-9 class” gene that contributes to the fertilization process in C. elegans. Mutations in this gene class cause animals to produce sperm with normal morphology and motility that cannot fertilize wild-type oocytes after contacting oocytes in the spermatheca. Loss of function spe-13 mutant hermaphrodites are sterile, and their fertility can be rescued by N2 males, suggesting that spe-13 is required in the sperm during fertilization. Like all spe-9 family mutants, spe-13 mutants undergo normal spermatogenesis and spermiogenesis (sperm activation). Mutants’ sperm accumulate in the spermatheca, yet still fail to fertilize the oocytes. To determine the spe-13’s gene sequence, we recently cloned spe-13 using Whole Genome Sequencing and found out that spe-13 is R06A10.5. SPE-13 is a small protein containing 130 amino acids with a predicted transmembrane domain and a large cytoplasmic tail. Our gene expression data showed that SPE-13 is exclusively expressed in sperm, indicating that SPE-13 is likely located on the sperm membrane required for fertilization. Through CRISPR/Cas9, we generated SPE-13-GFP strain to visualize the subcellular localization of SPE-13. Our finding confirmed our hypothesis that SPE-13 localizes to the cell membrane in the activated spermatozoa. Furthermore, by using a membranous organelles (MO) marker SPE-38, we conclude that SPE-13 localizes to the MOs in the spermatids. Finally, we found that for SPE-13 to correctly localize to the MOs in the spermatids and cell membrane in the spermatozoa, four other spe-9 class genes, spe-45, spe-9, spe-36, and spe-38, are required for these two separate steps, with spe-45 being required for the first step and the rest being required for the second step. Together, our results lead to the findings that SPE-13 is located on the sperm surface, playing a role in building the fertilization synapse complex on the sperm membrane for successful fertilization. In conclusion, our work identifies an additional component in the fertilization synapse in C. elegans and helps us better understand the complexity of the fertilization synapse. Similarly, small proteins have been identified in other species playing an essential role during fertilization, such as FIMP in mammals and Bouncer in zebrafish. Therefore, studying SPE-13 could also shed light on the roles

small proteins may play during fertilization.

1001T **The role of NUC-1 in apoptotic cell corpse clearance** Jonathan Pickett, Niccole Auld, Lathan Lucas, Omar Peña-Ramos, Xianghua Liu, Zheng Zhou Baylor College of Medicine

Approximately 300 billion cells die and need to be replaced in our bodies, daily. Degrading such vast amounts of cellular content, in addition to foreign material such as that of bacteria, requires the coordinated activity of specialized enzymes and subcellular compartments. One of the most important enzymes in this process, Deoxyribonuclease-2 (DNAse-2), is also responsible for preventing unnecessary immune responses to DNA. *nuc-1* is the *Caenorhabditis elegans* (*C. elegans*) gene that encodes the DNAse-2 enzyme. NUC-1 is primarily localized to lysosomes where the acidic environment allows this enzyme to function at its peak efficiency. We have developed a time-lapse imaging-based assay for monitoring the degradation of apoptotic cell corpse DNA in *C. elegans* embryos. Here, we show that NUC-1 acts in engulfing cell phagolysosomes to degrade apoptotic cell corpse DNA. The engulfment of apoptotic cells and lysosomal fusion to phagosomes, but not NUC-1 activity prior to engulfment, is necessary for NUC-1-mediated apoptotic cell corpse DNA degradation.

1002T **VAB-3/PAX6 regulates gonad morphogenesis in *C. elegans*** Victor Stolzenbach[1], Priti Agarwal[2], Ronen Zaidel-Bar[2], Erin Cram[1] [1]Biology, Northeastern University, [2]Cell and Developmental Biology, Tel Aviv University

The *C. elegans* gonad and distal tip cell (DTC) is a well characterized model of migration driven organogenesis. As the organ develops the DTC guides the proliferating germ cells pushing from behind as it elongates away from the body center along the ventral side, performs a 180 degree turn, and continues to elongate toward the body center along the dorsal side before finally becoming immobile as the organ takes its final shape. To better understand the genes involved in the latter part of this process, specifically the cessation of migration, a novel RNA-seq dataset was generated from DTC's isolated at L4 and early adult stages. Analysis of this data identified *vab-3*, an ortholog of the human PAX6, as a highly expressed gene in the L4 phase. Inhibition of *vab-3* via RNAi causes the gonad to continue to elongate well into adulthood resulting in a variety of swirl-like phenotypes. The cascade by which *vab-3* causes this to occur is unknown. Here we show that expression of *hlh-12*, a bHLH type transcription factor crucial for proper DTC migration, declines ~72 hours post egg laying. This coincides with the period of time when the DTC begins to halt migration as the organ finishes developing. In a *hlh-12* GFP model, expression of GFP is prolonged and increased significantly in *vab-3* RNAi conditions. These results suggest that *hlh-12* is central to maintaining the migratory programming of the DTC since loss of its expression correlates with cessation of migration.

1003T **Genetic evidence that the Pumilio-family proteins PUF-3 and PUF-11 repress SPN-4 translation in oocytes to prevent premature CCR4-NOT-mediated maternal RNA destabilization in *C. elegans*** Erika Tsukamoto[1], Micah Gearhart[2], Caroline Spike[2], David Greenstein[2] [1]Genetics, Cell Biology and Development, University of Minnesota, [2]University of Minnesota

Purified LIN-41-containing ribonucleoprotein complexes contain several different RNA-binding proteins, including the nearly identical and functionally redundant Pumilio-family RNA-binding proteins PUF-3 and PUF-11 (Tsukamoto et al., 2017). LIN-41 and PUF-3/11 promote the 3'UTR-mediated translational repression of the Rbfox-related RNA-binding protein SPN-4 in oocytes (Hubstenberger et al., 2012; Tsukamoto et al., 2017). Interestingly, the maternal-effect embryonic lethality caused by null mutations in *puf-3/11* is partially suppressed by mutations in *spn-4*. These observations suggest that SPN-4 over-expression in oocytes contributes to *puf-3/11* embryonic lethality. To understand the origin of *puf-3/11* maternal-effect lethality more completely, we screened 28,498 EMS-mutagenized haploid genomes for dominant suppressors of *puf-3/11* lethality and identified 24 strains in which *puf-3/11* lethality was suppressed enough to permit backcrossing. Whole genome sequencing was used to identify candidate suppressor mutants in each strain. As expected, we identified a new allele of *spn-4*; one of the strains was heterozygous for a maternal-effect-lethal allele of *spn-4* with a premature stop codon. More intriguingly, nine of the suppressed strains carried independent mutations in two different genes that encode essential subunits of the CCR4-NOT mRNA deadenylase complex: *let-711/*Not1 (8 strains) and *ccf-1/*Caf1 (1 strain). Most of these mutations were heterozygous in the suppressed strains and likely to be strong loss-of-function alleles. We have confirmed that other, independently-generated alleles of *let-711* and *ccf-1* dominantly suppress *puf-3/11* maternal-effect embryonic lethality. To test for more suppressors, we independently generated mutations in two of the other components of the CCR4-NOT complex: *ntl-2* and *ccr-4*. Our preliminary results suggest that mutations in *ccr-4* suppress *puf-3/11* maternal-effect embryonic lethality. Our current model suggests that the SPN-4 RNA-binding protein plays an important role in targeting specific mRNAs for deadenylation and decay during the oocyte-to-embryo transition. According to this model, in *puf-3/11* null mutants, SPN-4 is precociously expressed in proximal oocytes and causes premature clearance of maternal mRNAs with which it associates. We are currently trying to determine whether SPN-4 selects mRNAs for degradation by virtue of specific high-affinity RNA binding.

1004T **A Sperm–Oocyte Protein Complex as an Actin Regulator Required for Egg Activation in *Caenorhabditis elegans*** Tatsuya Tsukamoto[1], Ji Kent Kwah[2], Naomi Courtemanche[1], Andy Golden[3], David Greenstein[1], Aimee Jaramillo-

Lambert[2] [1]Department of Genetics, Cell Biology, and Development, University of Minnesota, [2]Department of Biological Sciences, University of Delaware, [3]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health

Fertilization triggers the completion of female meiosis and launches the oocyte-to-embryo transition. *C. elegans spe-11* is one of a few paternal-effect lethal genes. We report that SPE-11 forms a protein complex with an oocyte protein, OOPS-1 (Oocyte Partner of SPE-11), and that the complex is required for many post-fertilization events, including the proper completion of meiosis, the block to polyspermy, and eggshell formation. We discovered *oops-1* as an mRNA target of the oogenic LIN-41 and OMA-1 RNA-binding proteins. Tandem affinity purification (TAP) of OOPS-1 followed by mass spectrometry identified SPE-11. Likewise, SPE-11 TAP identified OOPS-1. Expression of both proteins in *E. coli* yields a stable complex, which we purified. Consistent with this molecular interaction, we determined that *oops-1* and *spe-11* exhibit apparently identical null phenotypes—approximately half of the mutant embryos display meiotic arrest in meiosis I or meiosis II, with the spindle drifting away from the cortex; whereas the other half display a phenotype in which meiotic chromosomes segregate at anaphase I and II but fail to form polar bodies. McNally and McNally (2005) noted that *spe-11* mutant embryos exhibit similarities to embryos treated with the F-actin inhibitor Latrunculin. Thus, we tested whether the OOPS-1–SPE-11 complex modulates actin assembly. Our experiments show that the complex binds F-actin in the absence of other proteins and inhibits formin-mediated actin assembly *in vitro*. Thus, the OOPS-1–SPE-11 complex may function in part by mediating key meiotic roles of F-actin. We are currently exploring this possibility using live-cell imaging. We isolated new mutations that suppress a temperature-sensitive *spe-11* mutant at the non-permissive temperature. Analyses of these new extragenic suppressor mutations might provide information on the mechanisms by which the OOPS-1–SPE-11 complex promotes the oocyte-to-embryo transition. We replicated the result that SPE-11 promotes embryonic development when ectopically expressed in the maternal germline (Browning and Strome, 1996). This finding raises the question of why evolution has separated OOPS-1 and SPE-11 into separate gametes. A potential answer is suggested by the observation that strains expressing SPE-11 in both male and female gametes exhibit transgenerational low fertility and slow growth phenotypes, indicating a potential role in paternal epigenetic inheritance.

1005T **The role of the HSP-90 co-chaperone SUGT-1 in GLP-1/Notch signaling and germline development in *C. elegans*** Jonathan C. Bastick[1], Eleanor M. Maine[2], James Lissemore[1] [1]Biology, John Carroll University, [2]Biology, Syracuse University

Activation of the GLP-1/Notch transmembrane receptor on the surface of the germline is required for germline stem cell maintenance and a number of other functions in *C. elegans*, including oocyte development and embryonic cell fate decisions. We have shown previously that the molecular chaperone HSP-90 promotes GLP-1/Notch signaling in the germline of *C. elegans.* In other systems, it has been shown that HSP-90 requires the assistance of a number of co-chaperones to conduct its protein-folding function. *C. elegans* encodes orthologs of ~17 HSP-90 co-chaperones, raising the question of which, if any, of these co-chaperones function in the GLP-1/Notch signaling pathway. We have been investigating the possible roles of HSP-90 co-chaperones in GLP-1/Notch signaling using RNA interference (RNAi) by feeding to knockdown expression of co-chaperone genes in N2 (wildtype) and *glp-1(bn18ts)* worms. *glp-1(bn18ts)* provides a sensitized background with reduced GLP-1/Notch signaling to reveal possible genetic interactions between GLP-1/Notch and HSP-90 co-chaperones. The SUGT-1 co-chaperone, encoded by the *D1054.3* gene, plays a role in kinetochore assembly and microtubule function in other systems, but there are little data on its function in *C. elegans*, and its possible role in GLP-1/Notch signaling has not been explored.

We have examined the effect of RNAi knockdown of *D1054.3* expression in N2 and *glp-1(bn18ts)*. Specifically, we have measured brood sizes and embryonic/larval lethality and have used DIC and fluorescence microscopy (with DAPI) to examine the germlines of adult worms. With respect to brood sizes, in N2 worms *D1054.3* knockdown had a variable effect with some animals producing broods of normal size (~250-300 embryos) and others being sterile. Sterility was low penetrance, ~25%. In contrast, knockdown of *D1054.3* had a stronger effect in *glp-1(bn18ts),* with ~75% sterility and no worms producing normal size broods. DIC and fluorescence microscopy revealed that, regardless of background, reduction of SUGT-1 function caused defects in oogenesis (oocyte shape and position), spermatogenesis (uncompacted sperm), and chromosome morphology in pachytene nuclei. Furthermore, in the *glp-1(bn18ts)* background, reduction of SUGT-1 function caused, in some animals, endomitotic DNA replication in oocytes (Emo phenotype), possible polyploid embryos, and large numbers of embryos retained (Egl phenotype). Taken together, our results suggest that SUGT-1 plays important roles in germline function and in GLP-1/Notch signaling in oogenesis.

1006T **The EBAX-type Cullin-RING E3 ligase promotes Linker Cell-type Death, a conserved non-apoptotic developmental cell death program.** Lauren Bayer Horowitz, Shai Shaham Laboratory of Developmental Genetics, Rockefeller University

Programmed cell death is essential for animal development and homeostasis, and its disruption is associated with many

disorders including neurodegeneration and cancer. Apoptosis is a prominent cell death form, however mutations in key apoptotic regulators cause only minor developmental defects in vertebrates. Non-apoptotic programs also exist, but their molecular basis remains poorly understood. Linker Cell-type Death (LCD) is a conserved non-apoptotic and caspase-independent cell death program that operates in *C. elegans* development. The Ubiquitin Proteasome System (UPS) is a key effector of LCD. The heat shock factor HSF-1 activates transcription of the E2 ubiquitin-conjugating enzyme LET-70/Ube2D2, which stimulates proteasome activity and effects LCD. The proteolytic targets of the UPS during LCD, however, and how their degradation triggers linker cell demise are unknown. In a screen for additional LCD regulators, we found that the conserved E3 ligase BC-box protein, EBAX-1, is required for LCD. EBAX-1 was previously shown to function as the substrate recognition subunit of Cul-2 based E3 ligases and to promote degradation of misfolded proteins. We hypothesize that EBAX-1 functions similarly during LCD to target specific substrates for ubiquitination. EBAX-1 is expressed in the linker cell throughout its lifetime and functions cell autonomously to promote LCD. We also show that EBAX-1 acts downstream of HSF-1 and LET-70 during LCD, suggesting that EBAX-1 functions with the UPS. To identify candidate substrates that EBAX-1 targets for degradation during LCD, we screened previously identified substrates of EBAX-1 and its homologs. Intriguingly, reduced expression of either of the homologs of the human argonaute AGO2, *alg-1* or *alg-2*, is sufficient to restore cell death to *ebax-1* mutant linker cells. Thus, ALG-1 and ALG-2 may be targeted for ubiquitination to promote LCD. In contrast to apoptosis where caspase effectors only transiently bind their targets, UPS E3 proteins can stably interact with substrates, providing an opportunity to identify the key cellular processes that are dismantled during cell death. The mechanisms we discover may, therefore, unearth general mechanisms of cellular destruction that also function during apoptosis and in disease.

1007T **Role of Dicer methylation during oocyte-to-embryo transition in *C. elegans*** Nick Newkirk[1], Shin-Yu Chen[2], Ryan Bell[3], Hongyuan Li[4], Mark Bedford[5], Andrew Z Fire[6], Swathi Arur[4] [1]MD Anderson UTHealth Graduate School of Biomedical Sciences, [2]Department of Genetics, MD Anderson, [3]Stanford University, [4]Department of Genetics, MD Anderson Cancer Center, [5]Department of Epigenetics and Molecular Carcinogenesis, MD Anderson Cancer Center, [6]Department of Genetics and Pathology, Stanford University

Dicer, a small RNA processing protein, is critical for regulation of gene expression. Misregulation of Dicer results in various diseases, infertility and developmental defects. Complete loss of Dicer, in worms and mice, while pleiotropic, results in failed oocyte formation and complete sterility. Due to its critical role in fertility and development, Dicer's activity is tightly controlled through post-translational modifications. While performing mass spectrometric analysis of human Dicer proteins, we identified a novel Arginine methylation site in the helicase domain of Dicer. *In vitro* methylation confirmed that this Arginine methylation is conserved from human to *C. elegans* Dicer. The goal of this study is to determine the effect of Dicer methylation, *in vivo*, using the model organism *C. elegans,* with a focus on its role in development and fertility. Using the combination of CRISPR mediated edits, live imaging, and biochemical analysis, we identify a specific role for Dicer methylation in completion of anaphase I of meiosis I during the oocyte-to-embryo transition. Defects in this process result in non-viable embryos rather than developing zygotes. Ongoing work to define the small RNA populations affected by this modification, and its methyl domain reader, will help define the molecular mechanism through which methylation of Dicer, on a conserved Arginine residue, regulates oocyte-to-embryo transition.

1008T **PP1cβ associated with two myosin phosphatase-targeting subunits protects against rupture during embryo elongation** Neha Varshney[1], Rebecca Green[1], Sindy Chavez[1], Pablo Lara-Gonzalez[2], Karen Oegema[1,3], Arshad Desai[1,3] [1]Department of Cell & Developmental Biology, University of California, San Diego, [2]Department of Developmental and Cell Biology, University of California, Irvine, [3]Department of Cellular and Molecular Medicine, University of California, San Diego

Protein phosphatase 1 (PP1) dephosphorylates a diverse set of biological targets to regulate several essential cellular processes in cells and tissues. The mechanisms that regulate PP1 specificity for different targets at distinct times and spatial locations is an active area of research. Here, we investigate the regulation of PP1 during embryonic development of *C. elegans*, when two catalytic isoforms, PP1cβ^{GSP-1} and PP1cα^{GSP-2}, are active. These two isoforms are ~90% identical, are expressed at similar levels, and function redundantly during early embryonic divisions. Double depletion of both isoforms leads to severe defects in the one-cell embryo. Despite redundant function in early embryonic divisions, depletion of PP1cβ^{GSP-1}, but not PP1cα^{GSP-2}, results in embryonic lethality, indicating that PP1cβ^{GSP-1} performs specific essential functions during the later stages of embryonic development that cannot be compensated for by PP1cα^{GSP-2}. Using a high-content imaging approach, we show that PP1cβ^{GSP-1} is essential for proper head enclosure and elongation during development. To investigate if PP1cβ^{GSP-1}-specific target subunits are also required for embryonic development, we screened candidate subunits from the myosin phosphatase (MYPT) family. Depletion of the myosin phosphatase targeting subunit 1 (MYPT1) homolog, MEL-11, phenocopied the effect of PP1cβ^{GSP-1} depletion. Surprisingly, knockdown of the *C. elegans* MYPT3 homolog GFI-2 also resulted in a similar, albeit less penetrant, rupture phenotype. Yeast 2-hybrid analysis indicates that MEL-11 and GFI-2 interact preferentially

with PP1cβ^{GSP-1} over PP1cα^{GSP-2}. To address which tissue and developmental processes require PP1cβ^{GSP-1}-MYPT activity, we are developing the means for inducible and efficient degradation in the skin, intestine, and body wall muscle. In addition to revealing how a specific PP1c isoform contributes to a key morphogenetic event during embryonic development, these efforts have the potential to provide mechanistic insight into the origins of a rare human congenital disease, Noonan-like syndrome, which is associated with mutations in PP1cβ.

1009T **Understanding the roles of a sperm-oocyte protein complex (SPE-11-OOPS-1) in *C. elegans* egg activation** Ji Kent Kwah[1], Tatsuya Tsukamoto[2], David Greenstein[2], Andy Golden[3], Aimee Jaramillo-Lambert[1] [1]University of Delaware, [2]University of Minnesota, [3]National Institutes of Health

The cornerstone of sexual reproduction is fertilization, where one sperm and one oocyte fuse, triggering embryonic development. In many animals, the sperm provides a haploid genome, a pair of centrioles for the first mitotic division, and the signal for egg activation. Mutants lacking these factors are known as paternal-effect embryonic lethal (PEL). In *C. elegans*, *spe-11* is the only known strictly PEL gene. Oocytes fertilized by sperm lacking SPE-11 have defects in egg activation and the egg-to-embryo transition resulting in embryonic lethality. The mechanism of SPE-11 in egg activation remains elusive. We identified a gene encoding an interacting partner of SPE-11 that is expressed in the oocyte; *oops-1* (oocyte partner of SPE-11). We found that OOPS-1 is localized to the cortex of oocytes. Characterization of a whole open reading frame (ORF) deletion mutant of *oops-1* [*oops-1(tn1898Δ)*] showed that, similar to *spe-11* mutants, *oops-1(tn1898Δ)* produces a severely reduced number of viable progeny. We imaged oocyte meiosis *in vivo* in wild-type, *spe-11(hc90)* [W191Stop], and *oops-1(tn1898Δ)* strains. Similar to previously published data, the chromosomes of *spe-11(hc90)* mutant embryos segregate at anaphase I and II but fail to form polar bodies. However, *oops-1(tn1898Δ)* mutants show various meiotic phenotypes with approximately half arresting at either meiosis I or II and the remaining mutant embryos displaying a *spe-11*-like phenotype. Like the *spe-11* mutants, polar body formation of the *oops-1* mutant is disrupted in examples that exit meiosis I. As previous genetic results suggested that *spe-11(hc90)* is a hypomorphic allele, we also analyzed *spe-11(tn2059Δ)* whole ORF deletion null mutants and found *spe-11(tn2059Δ)* had meiotic defects similar to *oops-1(tn1898Δ)* null mutants. In addition to meiosis, we are investigating the role of SPE-11 and OOPS-1 in the formation of the eggshell, which is essential for prevention of polyspermy and embryonic development. Both *spe-11(hc90)* and *oops-1(tn1898Δ)* mutants display similar defects in eggshell integrity. We found that *oops-1(tn1898Δ)* have defects in chitin formation similar to previous work in the *spe-11(hc90)* mutant. Analysis of strains harboring fluorescently labeled proteins involved in chitin layer formation in the eggshell (CBD-1 and CHS-1) and the egg-to-embryo transition (MBK-2), shows that the localization and expression of these proteins remain unchanged. Thus, the source of the eggshell defects when OOPS-1 and SPE-11 are compromised is still under investigation.

1010T **A sensory cilium mediates specific neuron-glia attachment** Leland Wexler[1], Max Heiman[2] [1]Genetics, Boston Childrens Hospital, [2]Harvard Medical School

Glial cells form specialized attachments with specific neuronal partners, but how such precise cell-cell pairings occur remains unknown. Here, we show that a sensory cilium mediates the highly stereotyped attachment of a single *C. elegans* neuron, called URX, to its partner glial cell, called ILso. The URX neuron offers a remarkable example of specificity in neuron-glia pairing. Its dendrite is positioned in the dorsal sensory bundle of the head and makes a “jump” across the nose tip to attach to a specific glial partner (ILso) in the lateral sensory bundle. Although 36 different glial endings are present at the nose tip, the URX dendrite recognizes and makes exclusive stereotyped attachments to the lateral ILso glial cell. We found that this specific neuron-glia pairing occurs by a multistep process. First, in early embryos, the URX dendrite anchors to a different glial guidepost, via the adhesion protein SAX-7 and the scaffolding protein GRDN-1, such that the dendrite is stretched out to its full length during embryo elongation. Mutants that disrupt dendrite anchoring result in severely shortened URX dendrites that fail to reach the nose tip. Second, in late embryos, we find that the URX dendrite ending jumps across the nose to form its mature attachment to the ILso glial cell. Using three-color imaging of URX, a cilia marker, and the ILso glial cell, we find that this attachment is mediated by a sensory cilium. By examining mutants that cannot form cilia, we find that the URX dendrite fails to attach to the lateral ILso glia, showing that the sensory cilium is strictly required for this neuron-glia attachment. Through a candidate screen we found that the loss of the conserved SPARC family protein TEST-1 results in the sensory cilium failing to attach to the glial partner. Importantly, vertebrate SPARC family proteins are secreted by glia and regulate adhesion at synapses, suggesting cilia-glia pairing may resemble pairing of dendritic spines and glia at vertebrate synapses. Finally, we used an unbiased forward genetic screen for mutants with defects in URX-ILso attachment and isolated several mutants that severely disrupt cilia-glia attachment. Together, our results show that, in addition to their canonical role in sensory signaling, cilia can mediate cell-cell adhesion including in the context of specific neuron-glia pairing.

1011T **The genetic interaction between *unc-33* and *hmp-2* in embryonic epidermal morphogenesis of *Caenorhabditis elegans*** Grace Gottschamer, Stephanie Maiden Biology, Truman State University

The embryonic epidermis in *Caenorhabditis elegans* is a simple genetic model to understand epithelial cell structure and

movement during development. Epidermal morphogenesis involves the intercalation of the dorsal-most cells, epiboly of ventrally-migrating cells, and epidermal elongation. These processes require the cadherin/catenin complex, specifically HMR-1/cadherin, HMP-2/β-catenin, and HMP-1/α-catenin proteins, to maintain cell-cell contacts and tissue integrity. In epidermal enclosure and elongation, α-catenin binds to actin, strengthening cell adhesions. Through drug treatments with microfilament and microtubule inhibitors, actin and microtubules have both been shown to be required for epidermal morphogenesis. More research has been conducted on the role of actin and its binding proteins than on microtubule binding proteins in epidermal morphogenesis. The *unc-33* gene encodes a microtubule regulator known for its role in neuronal development. Mutations in *unc-33* cause an abundance of microtubules and irregular axon outgrowth, which results in uncoordinated animal movement. We previously showed that knockdown of *hmp-2*/β-catenin by feeding RNA interference (RNAi) in homozygous *unc-33(e204)* animals results in 65.3% of embryos that failed to hatch compared to 17.9% in wildtype animals. Using differential interference contrast microscopy, we found that feeding *hmp-2(RNAi)* resulted in 100% of wildtype and 80% of homozygous *unc-33(e204)* embryos developing a humpback phenotype, and 0% of wildtype and 60% of homozygous *unc-33(e204)* embryos with rupturing of internal cells between ventral enclosure and two-fold body elongation. This suggests the epidermal contractions through actin tore the epidermal sheet that was not properly formed. Future experiments looking at participating microtubules and actin with fluorescent confocal microscopy would shed light on the genetic interaction between *unc-33* and *hmp-2*. The active muscle could also be putting further strain on the developing epidermis in *unc-33(e204);hmp-2(RNAi)* embryos, but this would require future analysis.

1012T **The role of phosphorylation of ZIPT-7.1 on *Caenorhabditis elegans* sperm maturation** Trace Ackley, Stephanie Maiden Biology, Truman State University

Spermatogenesis is the process by which germ line stem cells divide into haploid cells and differentiate into functional gametes. The latter portion of this process is sperm maturation, which is a rapid, multi-step pathway that leads to the motility of sperm cells. *Caenorhabditis elegans* has been used as a model organism to help understand the molecular mechanisms involved in this maturation process. *C. elegans* sperm maturation is a change in shape from a round immotile spermatid into a spermatozoa that contains a pseudopod which allows the sperm to crawl. For males, this rapid change in cell shape occurs when the seminal fluid is mixed with the spermatids during ejaculation. Although the entire process of sperm maturation has yet to be elucidated, a few steps have been determined essential for the process to occur. A large influx of zinc ions into the cytosol of the spermatid has been found to cause the formation of the pseudopod. In C. elegans, the process of moving zinc through the cell occurs through the ZIPT-7.1 transporter, which is a transmembrane protein found within membranes of certain vesicles that rapidly transport zinc ions from vesicles into the cytosol of the cell. The present project focuses on how ZIPT-7.1 may be activated via phosphorylation. Various bioinformatic tools were used to determine the possible phosphorylation sites of ZIPT-7.1. First, the protein sequence was plugged into the Net-Phos online computing system, which gives the mathematical probability of which amino acids may be phosphorylated. Then, the amino acid sequences of homologous ZIPT-7.1 proteins of mice, humans, zebra fish, and fruit flies were compared to the *C. elegans* sequence through a multiple sequence alignment tool, T-COFFEE. The amino acids with both the highest probability of phosphorylation and high conservation between homologs were chosen for targeted gene editing: Ser198Ala, Ser236Ala, Thr347Ala. Unlike serine and threonine, alanine cannot be phosphorylated. The genetic mutations in *C. elegans* will be introduced via CRISPR-Cas9 ribonucleoprotein complexes, and then sperm activation will be tested in stable lines. Thus far, control trials have indicated an 81% activation of wildtype spermatids using Pronase. If the mutated sperm do not activate as well as the wildtype, then the evidence would suggest that ZIPT-7.1 is regulated at those amino acids via phosphorylation.

1013T **Epidermal protein synthesis inhibition cell non-autonomously triggers organism-wide growth quiescence in C. elegans** Qiuxia Zhao, Rekha Rangan, Shinuo Weng, Cem Ozdemir, Elif Sarinay Cenik Molecular Biosciences, The University of Texas at Austin

Interorgan communication is crucial for multicellular organismal growth, development, and homeostasis. Here, we employed the auxin-inducible degradation system in C. elegans to temporally and spatially modulate protein translation, through depletion of essential factors (RPOA-2, GRWD-1, or TSR-2) for ribosome biogenesis. Our findings reveal that embryo-wide inhibition of ribosome biogenesis induces a reversible early larval growth quiescence, distinguished by a unique gene expression signature that is different from starvation or dauer stages. When ribosome biogenesis is inhibited in volumetrically similar tissues, including body wall muscle, epidermis, pharynx, intestine, or germ line, it results in proportionally stunted growth across the organism to different degrees. We show that specifically inhibiting ribosome biogenesis in the epidermis is sufficient to trigger an organism-wide growth quiescence. Epidermis-specific ribosome depletion leads to larval growth quiescence at the L3 stage, reduces organism-wide protein synthesis, and induces cell non-autonomous gene expression alterations. Furthermore, we find that UNC-31 and IDA-1, two dense-core vesicle (DCV) pathway components, play a significant role in epidermal ribosome biogenesis-mediated growth quiescence. Our tissue-specific knockdown experiments reveal that

the organism-wide growth quiescence induced by epidermal-specific ribosome biogenesis inhibition is suppressed by reducing unc-31 or ida-1 expression in the epidermis. Finally, we observe an overall increase in DCV puncta labeled by IDA- 1 when epidermal ribosome biogenesis is inhibited, and these puncta are present in or near epidermal cells. In conclusion, these findings suggest a novel mechanism of nutrition-independent multicellular growth coordination initiated from the epidermis tissue upon ribosome biogenesis inhibition.

1014T **SPE-54 is required for proper pseudopod shape and function in *C. elegans* sperm** Jack E Howell[1], Corinne S Vanella[2], Matthew Ragle[3], Lynn Zavada[2], Samuel Pasman[2], Zoe Johnson[3], Jordan Ward[3], Shantá Hinton[2], Diane C Shakes[2] [1]Biology, William & Mary, [2]William & Mary, [3]UC Santa Cruz

Unlike their flagella powered counterparts, nematode sperm crawl via pseudopod-based cell motility. Furthermore, in a distinct counterpart to actin-based motility systems, nematode sperm crawl via the polymerization/depolymerization dynamics of the Major Sperm Protein (MSP). Within the sperm's pseudopod, MSP assembles into non-polar filaments whose assembly dynamics are thought to be regulated by the worm's many sperm-specific phosphatases and kinases. Here we investigate the function of a non-receptor tyrosine phosphatase SPE-54, whose catalytic domain diverges from the canonical sequence. *spe-54* knockout mutants have dramatically reduced male fertility; males successfully inseminate females, but their sperm fail to migrate to the site of fertilization. Hermaphrodite self-fertility is partially impaired, with knockouts laying fewer embryos and higher numbers of unfertilized oocytes. As in wildtype males, *spe-54* males make spherical, haploid spermatids which activate to form polarized spermatozoa. During *spe-54* sperm activation, both membranous organelle (MO) fusion and MSP localization to the nascent pseudopod occur normally. However, the pseudopods of *spe-54* spermatozoa are both abnormally short and broad. Preliminary results reveal a reduction in pseudopod treadmilling rates that suggests a lowered activity of MSP depolymerization. To determine whether SPE-54's non-canonical catalytic domain impacts its phosphatase activity, we are testing eukaryotic-expressed SPE-54 for phosphatase activity. Further characterization of *spe-54* sperm motility defects and SPE-54's molecular function are ongoing

1015T **Exploring the role of SCF ubiquitin ligase in synaptonemal complex disassembly** Ailin Zhou, Yumi Kim Biology, Johns Hopkins University

Meiosis is a specialized cell division process essential for sexual reproduction. Unique to meiosis is its prolonged prophase, during which chromosomes pair and recombine with their homologous partners to ensure proper segregation. In most eukaryotes, this pairwise alignment between homologous chromosomes is mediated by a proteinaceous scaffold called the synaptonemal complex (SC). The SC forms a tripartite structure, consisting of two parallel chromosome axes and a central region that links the paired chromosomes. Our recent work identified the complete set of the SC central region components in *C. elegans*. Intriguingly, the final missing SC components are two Skp1-related proteins (SKR-1 and SKR-2), which are essential adaptors of the Skp1-Cul1-F-box (SCF) ubiquitin ligase that targets substrates for polyubiquitination-mediated protein degradation. The co-option of SKR proteins as SC subunits raises the possibility that the SC structure might protect its components from SCF-mediated polyubiquitination and subsequent degradation. However, it is unknown whether SCF targets SC proteins for proteolysis at the end of pachytene to allow its rapid disassembly. Here, we show that the levels of SC proteins are significantly reduced when they are not integrated into the SC due to the absence of one of its subunits. The stability of SC proteins is restored upon depletion of the RPN-3 subunit of the proteasome, indicating that SC proteins are subject to proteasome-mediated proteolysis when they are not part of the SC. Currently, we are investigating whether the degradation of SC proteins depends on the SCF activity and will present our latest findings.

1016T **Germline versus somatic stem cells: metabolism of distinct lineages composing the *Drosophila* ovary** Emily M Wessel[1,2], Daniela Drummond-Barbosa[1,2] [1]Genetics, University of Wisconsin- Madison, [2]Morgridge Institute for Research

Stem cells and their differentiating progeny have different metabolisms according to their distinct cellular states and functions. Few studies have examined the specific metabolic requirements along stem cell lineages in the context of whole living organisms, where complex tissues can house multiple stem cell types. In the *Drosophila* ovary, two very different stem cell lineages - derived from germline stem cells and somatic follicle stem cells - closely interact and are nutritionally regulated at various steps. Progeny of the germline stem cells undergo drastic differentiation to form a large mature oocyte while the follicle cells follow a more traditional somatic lineage. In this study, we examined the cell-autonomous requirements for fatty acid oxidation versus glycolysis in this model system using FLP/*FRT*-mediated genetic mosaic analysis. Remarkably, we found that fatty acid oxidation is not required in either germline stem cell or follicle stem cell lineages under normal conditions. By contrast, glycolysis is required for the maintenance of germline stem cells, for survival of follicle stem cells and their earliest progeny, and for the proliferation of more differentiated mitotically dividing follicle cells. Glycolysis is also required at later stages in the germline for egg chamber growth and survival, and also for normal nuclear development in both the germline and follicle cells. Surprisingly, the proliferation of germline stem cells and their early daughter cells does not require glycolysis,

suggesting an unusual metabolic state. Future studies will address the mechanistic basis for these specific requirements, and what alternative metabolic requirements are at play.

1017T **Looking for stress pathways modulated by heat stress during *Drosophila melanogaster* oogenesis** Ana Caroline Gandara[1,2], Daniela Drummond-Barbosa[1] [1]University of Wisconsin-Madison, [2]Morgridge Institute for Research

Temperature influences fertility across organisms; however, how suboptimal temperatures affect adult gametogenesis remains understudied despite the current climate crisis. We recently reported two in-depth analyses of how chronic exposure to suboptimal temperatures affects adult gametogenesis using *Drosophila melanogaster*. Male fertility was drastically reduced at 29°C (but not 18°C) due to low sperm abundance and quality. In females maintained at 18°C or 29°C, egg production was reduced (relative to 25°C control) through distinct cellular mechanisms: 29°C increased early germline cyst and vitellogenic follicle death and decreased oocyte quality, whereas 18°C slowed follicle growth. Surprisingly, germline stem cell (GSC) behavior and follicle growth were not affected in females at 29°C, raising the possibility of mechanisms partially protecting the germline. We are currently investigating the mechanisms responsible for female germline protection at 29°C using a combination of candidate and unbiased approaches. These studies will provide new insight into how temperature-dependent factors impact the female germline in a key model insect. Further, they will provide a foundation for a broader understanding of the effects of climate change on the reproduction of a wide range of species.

1018T **Innexins proteins regulate the breaking and making cell-cell interactions during collective cell migration** Guangxia Miao biological science, Florida State University

In the study of cell movement, much of the attention has been focused on observing how cells traverse from one location to another. However, there remains a substantial gap in the understanding of how cell collectives break away from their initial neighbors in the process of delamination. Even less is known about how cells make new connections upon arrival at their ultimate destination. To be concise I name this process neolamination.

I have established an *in vivo* model using the border cells in the *Drosophila* ovary to study both delamination and neolamination. Utilizing the powerful tools of *Drosophila* genetics, coupled with newly emerged optogenetics techniques and high-resolution live imaging, I have delved deep into the intricacies of collective border cell migration. In prior research, I investigated mechanisms by which border cells separate from the follicular epithelium in the process of delamination and how they make new connections upon reaching the oocyte, a process of neolamination. This research led to the identification of key stages and the regulatory genes involved in each process.

A standout discovery was the crucial role of innexins proteins, responsible for forming gap junctions between cells. These proteins facilitate the diffusion of ions and small molecules between cells. Intriguingly, we observed that during the neolamination process, innexins function in a channel-independent manner, partially through the regulation of microtubule (MT) abundance and its post-translational modifications. In this work, I will exploit our newly-developed live imaging to study the border cell MT dynamics to uncover how innexins regulate MTs. Innexins are also essential for delamination and I will compare and contrast their contributions to these two processes.

1019T **Investigating the roles of kinases and phosphatases in meiotic biorientation and spindle assembly** Madeline Terry[1], Janet Jang McKim[2], Manisha Persuad[2], Kim McKim[2] [1]Genetics, Waksman Institute of Microbiology, [2]Waksman Institute of Microbiology

During female meiotic cell division, the interactions between the kinetochores and the spindle are responsible for the correct segregation of chromosomes, independent of centrosomes. Correct genome partition relies on the chromosomes and their kinetochores establishing proper end-on attachments connected to microtubule fibers stemming from opposite spindle pole ends. The capture of spindle microtubules to kinetochores is error prone, which can lead to incorrect segregation of chromosomes. Unequal distribution of chromosomes can ultimately lead to spontaneous abortions, birth defects, and infertility. We are interested in determining the mechanisms for how kinases Aurora B, Aurora A, and Mps1 regulate error correction and end-on attachments to ensure the accurate biorientation of chromosomes during meiosis. Kinetochore subcomplex NDC80, recruited by SPC105R, is associated with the conversion of lateral to end-on attachments and is a target of Aurora B kinase during error correction. Aurora B, Mps1 and Aurora A localize to the central spindle, kinetochores and spindle poles respectively. Protein phosphatase 2A (PP2A) antagonizes the kinases and is necessary for NDC80 to establish stable end-on interactions with microtubules. How the kinases and PP2A interact to regulate biorientation, is not known. To better understand the relationship between the kinases and the shifting of attachments from lateral to end-on, we intend to confirm NDC80's ability to regulate end-on attachments through phosphomimetic and phospho-null mutants. To determine the mechanisms behind the kinases and their roles in spindle assembly, we intend to manipulate their locations by targeting the various kinases to either the spindle poles, central spindles, or the chromosomes, as well as generate knockdowns of spindle

assembly regulators. We will examine these phenotypes through confocal microscopy. We predict that NDC80 regulates end-on attachments and the kinases in three different locations are responsible for mediating correct microtube-kinetochore attachments.

1020T **The helicase activity of Me31B/DDX6 in *Drosophila* germline development** Evan Kara, Yousef Nammari, Raheem Mansoor, Deep Govani, Abraham Fielder, Brynn Nylin, Ming Gao Indiana University Northwest

Me31B (Maternally Expressed at 31B) is a putative ATP-dependent, RNA helicase essential for *Drosophila* female germline development. Our recent findings showed that mutations in Me31B's helicase domain led to female sterility in a dominant manner. However, the mechanistic role of the protein's helicase activity is not clear. In this study, we use Me31B-helicase-domain mutations (*me31B*^{E208A} and *me31B*^{DVLAAAA}) to investigate the role of the protein's helicase activity in the context of *Drosophila* germline. Previous research suggested that Me31B relies on its helicase domain to post-transcriptionally regulate germline RNA stability, translation, and decay. Therefore, we screened the two mutants for phenotypes in transcriptome (by mRNA/small RNA sequencing), proteome (by mass spectrometry), and translation of representative germline proteins (including Osk, Nos, Tral, Cup, and Me31B itself). The experiments revealed the mutants' phenotypes in oogenesis, embryogenesis, germ cell formation, protein localization, and so on. Our study indicates that Me31B's helicase activity is an integral part of Me31B protein function and plays an important role in *Drosophila* germline development.

1021T **Functional Role of Molecular Regulators in Determining Neuronal Morphology and Neurotransmitter Identity of TmY14 Subtypes in the Developing Drosophila Visual System** Maisha S Jacy, Claude Desplan, Yu-chieh D Chen Biology, New York University

The central nervous system has tremendous neurodiversity with different molecular and morphological distinct neuronal types. The molecular regulators controlling morphological features of different neuronal types remain to be elucidated. Combining a wide array of genetic toolkits and the whole brain EM connectome, the developing Drosophila visual system has been an excellent model system to address this important developmental question. Recent single-cell RNA sequencing (scRNAseq) data from the Desplan lab provides a comprehensive transcriptomic atlas for all optic lobe neurons of Drosophila throughout development. There are ~250 clusters, presumably 250 different cell types, identified by the scRNAseq analysis.

Although there are available genetics tools for target genetic manipulation in Drosophila, there is a lack of highly cell-type-specific targeting distinct cell types throughout development in the fly visual system. I firstly generated cell-type-specific split-GAL4 lines to label a transmedullatory neuronal (Tm) type throughout development: TmY14. Upon further examination of TmY14 neuronal morphology, I found two morphological subtypes with distinct lobula and brain projection patterns. I further examined the recently published EM connectome dataset and identified these two morphological subtypes of TmY14 with distinct neurotransmitter identities: one TmY14 subtype is predicted to be glutamatergic with projection to the central brain while another TmY14 subtype is predicted to be GABAergic without central brain projection. I aim to understand the molecular regulators underlying differences in the morphological and neurotransmitter differences in these two subtypes. Using the scRNAseq data, I will identify candidate genes differentially expressed in these two TmY14 subtypes and investigate their roles in regulating their morphological differences. Using the cell-specific split-GAL4 tool combining with Vglut-flp or Gad1-flp transgenes, I aim to knock down the differential expressed genes between glutamatergic and GABAergic TmY14 Cells and examine their roles in regulating subtype-specific neuronal features. The results of my project will shed light on the mechanisms underlying neuronal differentiation and allow further investigation of genetic regulation of neuronal morphological and functional features during development.

1022T **ex-vivo cultivation of Drosophila imaginal wing discs to study the mechanisms that determine whether cells live or die after radiation-induced caspase activation** Sarah I Colon, Tin Tin Su Molecular, Cellular and Developmental Biology, University of Colorado

Cells can survive after activation of apoptotic caspases, but what determines whether a cell lives or dies after caspase activation remains poorly understood. Taking advantage of a published reporter for past caspase activity (Ding et al., eLife, 2016; Sun et al., Nat Comm., 2020), we were able to visualize cells that survived caspase activation specifically after exposure to ionizing radiation in Drosophila larval wing discs. We found that cells with X-ray-induced past active caspases (XPAC) do not arise at random, but are born at specific locations within the developing imaginal wing discs of Drosophila larvae. Our data shows that the apoptotic signaling pathway is needed to induce XPAC cells as knocking down key components of the apoptotic pathway decreases XPAC number. Yet, XPAC cells appear in stereotypical patterns that do not follow the pattern of IR-induced apoptosis, suggesting additional controls at play. Functional testing identified the contribution of wingless (Drosophila Wnt1) and Ras signaling pathways to the prevalence of cells that activated caspases but did not die. To further study the influence of these two pathways in XPAC cells, ex-vivo cultivation of Drosophila imaginal wing discs has been conducted. Preliminary

data gathered shows that imaginal wing discs can remain alive in ex-vivo cultivation for 12 hours after exposure to radiation. However, tissues appear hypoxic at 12h post cultivation so further protocol optimization is needed. An ex-vivo system will allow us to test pharmacological inhibitors, such as inhibitors of Wnt and Ras signaling, and further understand how these pathways influence IR-induced caspase activity.

1023T **Investigating the role of the Ecdysone Receptor in germline to somatic cell communication** Lindsay Swain, Allison Simmons, Lauren Jung, Elizabeth Ables East Carolina University

Oogenesis requires complex coordination between both somatic and germ cell populations, relying on systemic signals such as hormones for proper oocyte production and formation. *Drosophila melanogaster* is an excellent model organism to explore how signaling from germ to somatic cells is required for viable egg production. *Drosophila* oogenesis utilizes steroid hormones such as ecdysone to facilitate many aspects of oocyte development such as germline stem cell proliferation, egg chamber growth, and border cell migration**.** Ecdysone function relies on reception by the heterodimeric complex, Ecdysone Receptor (EcR) and Ultaspiracle (Usp), that in turn activate transcriptional targets. EcR and Usp are expressed in both germline and somatic cell populations, but the role that ecdysone signaling plays in germ cells to control egg chamber development is unclear due to lack of suitable reagents to block ecdysone signaling specifically in germ cells. Here, we use novel reagents to deplete EcR levels or block transcriptional activation specifically in the germline. We find that depletion of EcR in germ cells results in a variety of somatic phenotypes. These include: an increased number of germaria with germ cell “collision” events, where cysts were not separated by follicle cells; shorter distance between cysts; and cysts with a longer aspect ratio. These phenotypes were accompanied by increased numbers of abnormal egg chambers, quantified as alterations in the number of oocytes per follicle, and slowed egg chamber growth. There was also a significant decrease in follicle cell proliferation, suggesting that EcR is needed to stimulate proliferation of overlying somatic cells. We also find that depletion of EcR in germ cells results in mild border cell migration phenotypes, such as delayed timing, cluster detachment, and altered cell number. Overall, these data suggest that in addition to its well-characterized roles in somatic follicle cells, EcR is necessary in the germline to promote timely egg chamber assembly and development.

1024T **Tnpo-SR promotes microtubule dynamics and fusome morphogenesis in *Drosophila* germline stem cells** Amanda Powell[1], Anna Williams[2], Lauren Anllo[1], Elizabeth Ables[2] [1]Biology, East Carolina University, [2]East Carolina University

Germline stem cells (GSCs) play a pivotal role in maintaining fertility across species as the precursors to oocytes and sperm, and also serve broadly as a model for how stem cells maintain their stemness. In *Drosophila* females, GSCs undergo asymmetric divisions that yield differentiating cystoblasts while maintaining the stem cell pool. The orientation of each asymmetric mitotic division is crucial for stem cell maintenance, yet the underlying molecular mechanisms governing this remain unresolved. One likely contributor to this establishment is the fusome, a cytoskeletal organelle with a tubulin-based core and endoplasmic reticulum-like membranous exterior. The fusome serves as a central hub for intracellular trafficking and facilitates GSC-cystoblast connectivity. We hypothesize that the fusome plays a functional role in re-establishing GSC polarity at each cell cycle. Interestingly a beta-importin, Tnpo-SR, seems to coordinate the maintenance of germline stem cells via the reorganization of microtubules. Loss of Tnpo-SR not only causes microtubule disorganization, mitotic misalignment, and fusome fragmentation but also leads to a loss of GSCs over time. We propose that by promoting proper microtubule dynamics, Tnpo-SR also promotes the intracellular movement of core fusome proteins back to the anterior pole of the stem cell, re-setting polarity at each division. This research highlights the significance that microtubules and cytoskeletal polarity play in maintaining stem cell self-renewal and identifies Tnpo-SR as a potential regulator of this process.

1025T **Coordination of cell cycle and morphogenesis during organ formation** Jeffrey Matthew, Vishakha Vishwarkarma, Thao P Lee, Ryan A Agsunod, SeYeon Chung Biological Sciences, Louisiana State University, LSU

Organ formation requires precise regulation of cell cycle and morphogenetic events. Using the Drosophila embryonic salivary gland (SG) as a model, we uncover the role of the SP1/KLF transcription factor Huckebein (Hkb) in coordinating cell cycle regulation and morphogenesis. The hkb mutant SG exhibits defects in invagination positioning and organ size due to abnormal death of SG cells. Normal SG development involves distal-to-proximal progression of endoreplication (endocycle), whereas hkb mutant SG cells undergo abnormal cell division, leading to cell death. Hkb represses the expression of key cell cycle and pro-apoptotic genes in the SG. Knockdown of cyclin E or cyclin-dependent kinase 1, or overexpression of fizzy-related rescues most of the morphogenetic defects observed in the hkb mutant SG. These results indicate that Hkb plays a critical role in controlling endoreplication by regulating the transcription of key cell cycle effectors to ensure proper organ formation.

1026T **Chondroitin sulfate is required for proper control of *Drosophila* intestinal stem cells** Collin Knudsen, Hiroshi Nakato University of Minnesota

Chondroitin sulfate (CS) and heparan sulfate (HS) are evolutionary conserved glycosaminoglycans found in most animal

species, including *Drosophila*. CS and HS exist as proteoglycans (PGs) in which the sugar polymers are covalently attached to core-proteins. While there have been substantial amounts of research conducted on the functions of *Drosophila* HSPGs, roles of CSPGs in *Drosophila* development remain to be elucidated. To investigate the function of CS in development and regeneration, we generated mutants for *Chondroitin sulfate synthase* (*Chsy*), which encodes the *Drosophila* homologue of mammalian Chondroitin synthase 1, a critical CS biosynthetic enzyme, via CRISPR-Cas9 mutagenesis. In the midgut, the loss of *Chsy* resulted in elevated levels of intestinal stem cell (ISC) division during homeostasis, leading to an abnormally increased thickness of the midgut. *Chondroitin polymerizing factor* (*Chpf*) was investigated during midgut homeostasis, with *Chpf* mutants also showing increased ISC division and midgut thickness. Chpf in mammals is known to bind to Chsy, helping to synthesize elongated CS. Additionally, the longitudinal visceral muscle of the midguts in *Chsy* mutants was altered, with significant increases in muscle discontinuity and sprouting in comparison to wild-type. The source of CS in the midgut was found to be from local visceral muscle and enterocytes. Using a regeneration model, we found that *Chsy* mutant ISCs failed to properly downregulate mitotic activity at the end of regeneration. These data showed that CS is required for proper control of the ISC mitogenic activity during midgut homeostasis and regeneration, and a loss of CS results in disrupted midgut morphology.

1027T **_Sar1_, a GTPase involved in COPII vesicle trafficking, is critical for _Drosophila_ oogenesis** Makayla C Gomperts, Julie A Merkle Biology, University of Evansville

The process of cell fate determination is a major question in developmental biology, and the current understanding of mechanisms by which it occurs is very limited. In *Drosophila melanogaster*, oogenesis, or egg production, begins with the division of germline stem cells, eventually leading to the formation of a cyst composed of 16 cells. From here, one is selected to become the future egg, while the other 15 become supporting nurse cells. An EMS mutagenesis genetic screen in *Drosophila* identified several mutations in genes that lead to defects in oocyte determination, ultimately leading to a loss of eggs and fertility. *Sec24CD*, which forms the inner part of the vesicle coat involved in COPII anterograde trafficking, was identified from this screen. Since this was the first known role for COPII trafficking in oogenesis, we were interested in investigating whether other COPII components were also involved in oogenesis. *Sar1* is the GTPase needed for coat assembly of COPII vesicles. To study and analyze the role of *Sar1* in oocyte determination, RNAi knockdown of *Sar1* was performed using a variety of germline-specific Gal4 drivers. This resulted in rudimentary ovaries lacking recognizable germaria or egg chambers, demonstrating that *Sar1* is required early in oogenesis. However, given the dramatic phenotype observed, investigation of *Sar1* in oocyte identity could not be assessed. Using *Gal80^{ts}* to restrict RNAi knockdown to limited windows during oogenesis, intermediate defects have been obtained, wherein egg chambers are made but lack oocytes. Available CRISPR alleles were also utilized to determine loss of function phenotypes of *Sar1* by employing the TRiP-KO and WKO systems. Co-IP experiments have been used to determine the interactions of *Sar1* with COPII vesicle components *Sec24CD* and *Sec23.* Preliminary data confirm interaction of *Sec24CD* and *Sec23* in ovary lysates. Altogether, investigating the role of *Sar1* and COPII vesicle trafficking will allow for better understanding of the molecular mechanisms that control oocyte fate determination in *Drosophila*.

1028T **Nucleoporin107 is a critical determinant of soma-germline communication, essential for ovarian development and function** Merav Yaffa Gold[1], Tgst Levi[1], Tikva Shore[1], Tzofia Bialistoky[1], Shira Leebhoff[1], Girish Deshpande[2], Offer Gerlitz[1] [1]Developmental Biology and Cancer Research, The Hebrew University, [2]Department of Molecular Biology, Princeton University

In earlier studies aimed at uncovering novel components of ovarian development, we identified a novel missense mutation in Nucleoporin107 (Nup107; D447N), an essential component of the nuclear pore complex, as the cause of XX-Ovarian Dysgenesis, a rare disorder characterized by underdeveloped and dysfunctional ovaries. We have employed Drosophila to model the specific human mutation. Either the expression of a mutant isoform or specific knockdown of Nup107 in the gonadal soma resulted in female-specific infertility due to aberrant ovarian development, which to a large extent mimicks the human phenotype. Our data demonstrated that Nup107 regulates essential soma-germline interactions in both larval and adult stages essential for ovarian differentiation and function. Comparative transcriptomic analysis on Nup107 mutant and control larval gonads identified 82 differentially regulated genes including the transcription factor *doublesex* (*dsx*), known for its critical role in sex-specific differentiation. Either loss or gain of Dsx in the gonadal soma is sufficient to mimic or rescue the phenotypes induced by *Nup107* loss, thus establishing Dsx as the primary target and effector of Nup107. Further analysis revealed the primary larval and adult somatic cell types in which the activity of Nup107 is necessary and have begun to elucidate the affected signaling pathways and their involvement in germline–soma communication. Our data thus demonstrate that a sex- and tissue-specific regulation of Dsx by a 'housekeeping' nuclear envelope protein Nup107 is crucial for the ovarian development and function. Given the impressive conservation of Nup107 and the similar phenotypes associated with the mutation, many of our findings will be relevant to human health and hopefully will advance our understanding and treatment of conditions pertaining to female infertility.

1029T **The T-box transcription factor Org-1 is required to establish the *Drosophila* testis niche.** Tynan Gardner[1], Patrick Hofe[2], Kirklan Naumuk[2], Stephen DiNardo[1], Lauren Anllo[2] [1]Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, [2]Biology, East Carolina University

Stem cells are required for tissue homeostasis and regeneration. Accomplishing such tasks often requires intimate association between stem cells and their cellular microenvironment, or niche. Unfortunately, we know little about how niches are initially established within tissues during organogenesis. We use the developing *Drosophila* testis as a model to study the genetic control of niche formation. Before niche assembly, the spherical gonad contains an interwoven mix of somatic cells encysting germ cells. A subset of these somatic cells is specified as pro-niche cells by Notch signaling prior to gonad coalescence (Kitadate & Kobayashi, 2010; Okegbe & DiNardo, 2011). Our imaging revealed that pro-niche cells extend protrusions to pull themselves out to the gonad periphery, where they migrate along extracellular matrix toward the anterior. We found that anterior assembly of the niche requires signaling from adjacent visceral muscle (Vm). In response to signaling, niche cells express the transcription factor *islet*, which is also required for positioning the niche (Anllo & DiNardo, 2022). Identifying regulators of *islet* expression would identify key intermediaries involved in niche assembly. To this end, we have shown that a minimal element from the *islet* enhancer containing Org1 binding sites is sufficient to drive gene expression in niche cells. *org1* is a T-box transcription factor with a known role regulating *islet* to specify embryonic muscles (Boukhatmi et al., 2014). Here, we show *org1* expression in embryonic niche cells, and significantly reduced Islet in the absence of *org1,* suggesting that Org1 mediates *islet* expression in the testis niche. Like *islet* mutants, *org1* mutant niche cells fail to form a single organized cluster at the anterior and are instead dispersed throughout the gonad. In addition, expression of certain key adhesion proteins is disrupted without *org1*: significantly fewer niche cells express N-cadherin, and all niche cells fail to express Fas3, suggesting additional function for *org1* in the forming niche beyond inducing *islet*. Future experiments will identify whether *org1* plays a dual role in regulating niche specification along with anterior cell movement. This work will enlighten our perspective of mechanisms that initially establish a compartmentalized stem cell niche during organogenesis.

1030T **Over-expression of the Ecdysone Receptor promotes an undifferentiated transitional state during germline stem cell differentiation** Lauren Jung, Alexandria Warren, Elizabeth Ables Biology, East Carolina University

The *Drosophila* ovary requires a tightly regulated network of signals to coordinate the division of their stem cells and differentiation of their daughter cells to produce viable oocytes. The steroid hormone ecdysone is known to be involved in germline stem cell (GSC) self-renewal, however its direct and autonomous role in the germline has not been evaluated. Ecdysone elicits a diverse array of transcriptional responses by binding to a heterodimeric complex composed of Ecdysone Receptor (EcR) and Ultraspiracle (Usp). To illuminate whether EcR facilitates autonomous reception of ecdysone in the germline, we built germline-compatible genetic tools to manipulate EcR levels and/or activity. We find that over-expressing either EcR.A or EcR.B1 isoforms in germ cells results in undifferentiated germ cell tumors and decreased numbers of BMP-responsive GSCs. Germ cells over-expressing EcR remained mitotic, failed to form cysts, and aggregated into egg chambers, reminiscent of cells with over-active BMP receptors or depleted *bam* levels. In tumorous ovaries, stem cell-like cells were also identified outside of their normal stem cell niche, suggesting that these cells remain competent to respond to BMP signals. We demonstrate that the tumor phenotype is dependent on ecdysone, as over-expression of an EcR.B1 transgene that cannot bind ligand suppresses tumor development. Moreover, restricting over-expression of EcR to more differentiated germ cells in 8- and 16-cell cysts failed to produce the tumor phenotype. These data suggest that EcR is required in the GSCs and their immediate daughters for the differentiation of cystoblasts and demonstrate that the low levels of EcR expressed in GSCs are sufficient for ligand-dependent activation of an ecdysone-responsive transcriptional program.

1031T **Cytokine Dynamics in Polyploidization During Drosophila Hindgut Regeneration** Paulo Belato, Donald Fox Pharmacology and Cancer Biology, Duke University

The ability to repair an injured tissue is a crucial part of survival for any organism. Although we usually think of regeneration through the lens of hyperplastic proliferation of tissue specific stem cells, many adult tissues lack these multipotent cells. As a result, one newly appreciated mechanism by which post-mitotic tissues can repair themselves after injury is by becoming polyploid. Polyploidy serves to create more genome copies and enlarge the cell, and thus can play an important role in tissue injury recovery. The fly pylorus is an excellent model to study wound-induced polyploidization because this diploid tissue lacks stem cells and relies on hypertrophic regeneration by endocycles (S-phases without M-phases). Incredibly, the remaining pyloric cells that survive injury are able to restore the tissue's cellular ploidy to match the number of genomes in the tissue pre-injury, irrespective of the injury severity (Cohen et al, 2018). As a clue to how polyploidy is tuned during pyloric regeneration, the Fox Lab has identified the JAK/STAT cytokine *upd3* as the molecular cue that signals injury and prompts remaining cells to engage with the endocycle. How *upd3* dynamically controls the regeneration program under various degrees of injury is poorly understood. To further this work, we are conducting high resolution quantitative imaging of an *upd3* reporter during pyloric regeneration. In preliminary experiments, we have observed that differences in pyloric injury

severity influence both *upd3* signal duration and amplitude in regenerating cells. In addition, we have also observed that the positional identity of pyloric cells along the anterior-posterior axis also affects the amplitude and duration *upd3* signaling in response to injury. Uncovering how regeneration-specific signaling dynamics start, monitor, and stop endocycling will reveal the factors involved in wound-induced polyploidization. Broadly, these findings can deepen our understanding of regeneration in tissues without constitutively active stem cells.

1032T **Dilp8 functions as a mature follicle sensor to prevent excessive accumulation of mature follicles in *Drosophila* ovaries.** Rebecca Oramas, Katarina Yacuk, Baosheng Zeng, Jianjun Sun Physiology and Neurobiology, University of Connecticut

Excessive accumulation and prolonged storage of mature follicles in *Drosophila* female ovaries leads to an unnecessary energy expenditure and oocyte aging, which affects the oocyte's quality and consequently decreases offspring hatching rates. As such, the number of mature follicles in *Drosophila* ovaries are tightly controlled. Each ovary is comprised of ~16 ovarioles and each ovariole contains 1-2 mature follicles, regardless of female's mating status. The mechanism by which the ovary counts the number of mature follicles remains a mystery. Previous work, and our recent RNAseq and antibody analysis, demonstrated that *Drosophila* insulin-like peptide 8 (DILP8) is specifically expressed in follicle cells of mature (stage-14) follicles but not in younger follicles. Contrary to previous findings, we found that mature follicle DILP8 is not essential for mating-induced ovulation and egg laying in an optimal condition. In contrast, we are surprised to find that DILP8 mutant females accumulate excessive mature follicles in their ovaries when the condition is not optimal, particularly when they are virgins. This also occurs when DILP8 is only knocked down in follicle cells. Considering the expression of DILP8 in mature follicle cells, we propose that DILP8 functions as a mature follicle sensor to regulate the number of mature follicles in the ovaries of virgin females. Further analysis indicates that DILP8 is required to induce ovulation and egg laying in virgin females to prevent excessive accumulation of mature follicles and oocyte aging. This mechanism may be very important in the wild, where females do not always have access to a male partner to cause mating-induced ovulation. The DILP8-mediated control of mature follicle number will ensure female maintain high quality oocytes all the time before the next mating.

1033T **Determining the requirement for PIP2 in *Drosophila melanogaster* sperm head-tail coupling** Marynelle S Icmat[1,2], Negar Nasirzadeh[1,2], Julie A Brill[1,2] [1]Department of Molecular Genetics, University of Toronto, [2]Cell Biology Program, The Hospital for Sick Children

The production of mature sperm from an undifferentiated germ cell is a highly regulated process involving dramatic morphological changes to form sperm heads and tails. The process by which the sperm tail is attached to the head, known as "sperm head-tail coupling", is an important phenomenon during sperm development. Since the development of fly and mammalian sperm is similar, we study this process using *Drosophila melanogaster*. In both mammalian and fly systems, sperm head-tail coupling requires the basal body, a specialized centriole that templates the sperm tail and functions as a microtubule organizing centre to promote nuclear elongation. A crucial step is anchoring of the basal body to the nuclear envelope, a process aided by centrosomal proteins. Flies with reduced levels of the lipid phosphatidylinositol 4,5-bisphosphate (PIP2), which is synthesized by the PIP5-kinase Skittles (Sktl) in Drosophila, are male sterile. Exactly how Sktl and PIP2 regulate sperm development remains unclear. In PIP2-depleted sperm, the basal body is initially anchored to the nuclear envelope. This attachment is lost as the nuclei continue to elongate, resulting in malformed sperm heads and sperm decapitation. Interestingly, reduced PIP2 levels result in altered recruitment and localization of centrosomal proteins known to be important for this process. These defects are rescued by over-expression of Sktl, indicating essential roles of Sktl and PIP2 in these processes. Furthermore, by combining mass spectrometry with BioID, a proximity-dependent labelling technique, we have discovered putative Sktl protein interactors during sperm development. Ongoing experiments will uncover regions of Sktl important for sperm development as well as identification of novel protein interactors that, through direct or indirect interaction with Sktl, mediate sperm head-tail coupling and other processes of sperm development.

1034T **Asperous, an EGF-Repeat Protein, Orchestrates Compartment-Specific Regenerative Growth in Drosophila Wing Discs** Si A Cave, Robin E Harirs Molecular Sciences, Arizona State University

Regeneration is a fundamental process that allows organisms to renew and replace damaged tissues. In this study, we utilize the *Drosophila* wing imaginal disc as a model system to unravel the mechanisms of an uncharacterized protein specifically involved in tissue regeneration.

Through RNA-sequencing, we identified *asperous* (CG9572) as a gene significantly upregulated during the regenerative process. Asperous (ASPR) presents a distinctive structural composition, featuring a signal peptide and repeating epidermal growth factor (EGF) domains that collectively form a WD40 structural motif. This structural arrangement prompts the hypothesis that ASPR is conceivably involved in intercellular signaling and the coordination of multi-protein complexes.

To decipher the specific function of ASPR, we employed Duration and Location (DUAL) Control, a novel genetic ablation system that induces cell death via apoptosis or necrosis while concurrently manipulating neighboring tissue. Our results demonstrate the indispensability of ASPR in regeneration following apoptotic cell death. Remarkably, reducing ASPR expression in the surrounding tissue after necrotic damage enhances regeneration, revealing a diverse role dependent on the nature of the tissue ablation.

Furthermore, ectopic expression of ASPR surprisingly hindered growth in a compartment-specific manner. Assessments of both the imaginal wing discs and wings following ablation revealed diminished anterior growth upon ASPR overexpression. Additionally, the restoration of anterior-specific markers observed during regeneration was notably absent in the presence of elevated ASPR levels, indicating a possible role in modulating cell identity post-ablation.

The expression levels and localization of ASPR appear to be critical determinants, influencing whether ASPR promotes or impedes regenerative growth.

This research underscores the importance of elucidating ASPR's compartment-specific role in different regenerative contexts. Its dual nature, dependent on the cell death type and spatial context, positions ASPR as a compelling target for future research. With its multifaceted role in tissue regeneration, ASPR promises valuable insights into the intricate mechanisms governing this fundamental biological process, with potential applications in advancing the understanding of compartment-specific growth patterns in regenerative biology.

1035T **Role of Malvolio, the *Drosophila* ortholog of human NRAMP2 metal ion transporter, in salivary gland morphogenesis** Rajprasad Loganathan[1], Srihitha Akula[2], Aditi Kulkarni[2], Tony Zhu[2], Rika Maruyama[2], Deborah Andrew[2] [1]Biological Sciences, Wichita State University, [2]Cell Biology, Johns Hopkins University

Malvolio (Mvl) is the Drosophila ortholog of the mammalian Solute Carrier Protein Slc11a2, which transports divalent metals, including iron. Mvl shows Fkh-dependent expression during embryonic salivary gland (SG) morphogenesis. *Mvl* zygotic loss has no effect on viability although a developmental delay was observed in $Mvl^{exc1}/Df(Mvl)$, with adults eclosing 48 – 72 hours later than their balancer-carrying siblings. Zygotic loss of *Mvl* also resulted in mild SG abnormalities and occasional small gaps in the denticles. Combined maternal and zygotic loss led to pronounced defects in SG morphology and high frequency denticle gaps. We also observed a loose assemblage of CrebA+ embryonic cells in the anterior region of the *Mvl* homozygotes that may be crystal cells (a subpopulation of Drosophila immune cells). Collectively, these results demonstrate varying degrees of developmental defects with the loss of *Mvl* during embryogenesis. Imaging of *Mvl* null embryos revealed that although the levels and localization of the adherens junction protein E-cadherin and polarity marker Bazooka were comparable to wild-type SGs, the levels of the apical polarity determinant Crumbs were notably decreased. To learn where Mvl localizes in SG cells and is likely to function, we generated Mvl antiserum and costained SGs with anti-Mvl in combination with several organelle-specific markers. These experiments revealed Mvl localization to Golgi, early, and late endosomes. We are testing a working hypothesis that the cell morphogenetic defects in *Mvl* loss-of-function are linked to defective endomembrane trafficking that affects Crb localization and recycling at the sub-apical domain. We are currently assaying the requirements for iron versus proton transport in *Mvl* function.

1036T **Effects of Developmental Alcohol Exposure on Parkinson's Disease models in Drosophila melanogaster** Navneet Sanghera Department of Biological Sciences, San Jose State Univeristy

We have established a Drosophila model of Fetal Alcohol Spectrum Disorder (FASD), a highly variable disorder that results from the consumption of alcohol during pregnancy. Exposing fetuses to alcohol during development can lead to effects including but not limited to developmental delays, low body weight, intellectual disabilities, behavioral changes, and reduced brain size. We have replicated most of these effects in Drosophila exposed to alcohol during development.

We carried out a large-scale genetic screen for mutations that alter the sensitivity of flies to developmental alcohol exposure (DAE). Through this screen, we found that mutations in the gene Dementin (Dmtn), which encodes the Drosophila ortholog of the Alzheimer Disease associated protein TMCC2, result in sensitivity to DAE. Using quantitative RT-PCR and locomotion assays, we showed that DAE results in changes in Dmtn expression, and that adult flies, after exposure to DAE, show climbing defects indicative of CNS dysfunction. Such climbing defects are also seen in fly models of neurodegenerative diseases (including Alzheimer's Disease).

Neurodegenerative diseases attack the central nervous system, which can lead to deficiencies in movement, power, senses, and cognition. We hypothesize that DAE causes neurodegeneration (and may target known pathways involved in neurodegeneration). Currently, we are surveying the interaction between DAE and Drosophila models of neurodegeneration specifically focused on mutants associated with proteins involved in Parkinson's Disease. We will present the results of rearing

Pink and Park mutant flies in ethanol. These two proteins work together in mitochondrial quality control by tagging damaged mitochondria with ubiquitin for further degradation. Interestingly, our results suggested an inverse relationship between these two mutants when exposed to alcohol during development. The park mutants were highly sensitive, while Pink1 mutants were resistant to the effects of alcohol exposure. In addition, we have performed climbing assays to test the health of the CNS system and our analysis indicates that DAE exacerbates climbing defects in young park mutants, but this effect does not worsen over time (suggesting an effect on CNS development).

We will also present data examining the interaction between DAE and climbing ability in Pink1 mutants results from survival assays on other Parkinson's disease associated mutants, and brain imaging to examine dopaminergic neuron degradation in Parkinson's Disease mutants reared on ethanol media.

1037T **Distant wound mediates gut homeostasis through blood cells in flies** Sveta Chakrabarti, Sandhya S Visweswariah Indian Institute of Science

Tissue damage triggers a complex response in animals, involving the activation of multiple pathways to repair the damage and protect against infection. This response relies on the detection of damage-associated molecular patterns, which signal cellular stress. Our recent study in flies has revealed that hydrogen peroxide, produced by blood cells, plays a critical role in the activation of immune pathways following injury. We also found that injured flies are better protected from infection with bacteria, suggesting that injury can train the immune system. Further research is needed to understand the molecular mechanisms by which hydrogen peroxide signals injury and activates immune responses. The gut plays a crucial role in immune function and nutrient absorption. Our current research demonstrates that blood cells relay injury signals to the intestine, stimulating stem cell proliferation and maintaining gut barrier integrity. We show here that the absence of hemocyte activation by ROS at the site of injury leads to a loss of adherence junctions in the gut leading to intestinal barrier dysfunction and increased lethality following injury. UPD3 produced from hemocytes activates STAT in the gut following injury, and chronic STAT activation in the intestine is required for the proper turnover of enterocytes post-injury and survival. Collectively, our results reveal that hemocytes remotely integrate signals to control intestinal homeostasis following an injury.

1038T **Investigating the effects of RNA binding protein interactions and RNAi knockdown phenotypes on *Drosophila* sperm development and mRNA localisation** Dana E Jackson, Sonia Lopez de Quinto, Helen White-Cooper Molecular Biosciences Division, School of Biosciences, Cardiff University

In *Drosophila melanogaster*, male germline cells advance through sperm development in a tightly regulated program of synchronised displacement, differentiation, elongation and individualisation.

While the bulk of spermatogenesis-specific transcription occurs in pre-meiotic primary spermatocytes, an exclusive subset of genes are also post-meiotically transcribed in mid-to-late elongating spermatids – at a point preceding the histone-transition protein-protamine switch. These genes express a number of asymmetrically-localised mRNAs that accumulate towards the distal tail-ends of growing spermatid cyst bundles; arranging in unusual localisation patterns of shooting speckled "comets" or U-shaped acorn "cups". Analysis of fluorescently tagged reporter constructs have revealed that this mRNA localisation precedes the formation of distinct protein gradients.

In addition to these localised mRNAs, we have also identified several RNA binding proteins (RBPs) enriched at the spermatid tail-ends. How and why do these non-uniform, subcellular mRNA distributions arise? What role do these RBPs play in regulating the active localisation, anchoring and translation of comet and cup mRNAs in spermatids?

We have used RNA-affinity pull-down assays to extract out bound fractions of comet and cup mRNAs with a pool of endogenous RBPs, including the highly-conserved IGF-II mRNA-binding protein (Imp). This revealed differential binding between 4 candidate RBPs and 11 comet and cup mRNAs *in vitro* – with binding affinities varying depending on the mRNA of interest. We are now in the process of optimising a modified Cl-AP protocol to precipitate whole, multi-RBP complexes (and their respective interacting RNAs) to re-verify these binding interactions in a more testis-authentic context, and to identify further co-localised mRNAs.

We have also performed a series of RNAi screens to investigate the functional roles of these RBPs in spermatids. We have found that a knockdown of *imp* leads to a variable spectrum of abnormal testis phenotypes, including mislocalisation of mRNA transcripts, loss of localised RNA and fluorescent protein reporter signals, and considerable disruption to spermatid elongation. By conducting HCR RNA-FISH and Lightsheet Fluorescence Microscopy, we have preliminarily determined that these RNAi-induced defects are detectable at both the level of translational dysregulation and at an earlier point of mRNA production, stability and/or localisation.

1039T **The Role of GATA Transcription Factor Serpent in Programmed Cell Death in *Drosophila* Ovaries** Baosheng Zeng, Haley Grayson, Jianjun Sun Physiology and Neurobiology, University of Connecticut

Programmed cell death (PCD) is a vital process for the survival of all living organisms. Recent research has revealed its essential roles in various stages of oogenesis, such as the degradation of mid-stage egg chambers and the developmental death of nurse cells in *Drosophila* ovaries. The GATA transcription factor Serpent (Srp) plays an important role in macrophage development and is crucial for phagocytosis in the embryo; however, its involvement in the cell death process within *Drosophila* ovaries has never been explored. In this study, we demonstrate that Srp is essential for proper cell death during oogenesis. Specifically, Srp becomes gradually upregulated in the follicle cells of degrading mid-stage egg chambers. *srp* knockdown results in premature death of follicle cells in degrading mid-stage egg chambers, leading to an incomplete clearance of dying germ cell debris. Molecular analysis indicates that *srp* knockdown leads to reduced expression of the engulfment receptor Draper (Drpr) in follicle cells and impairs the lysosomal activity in germ cells of degrading egg chambers. Our preliminary analysis indicates that Srp potentially acts downstream of the JNK pathway for the Drpr upregulation. Additionally, we found that Srp's role in cell death is not limited to mid-stage egg chambers, but also in the developmental death of nurse cells at the end of oogenesis. *srp* knockdown in follicle cells results in a lack of nurse cell acidification and persistent nurse cell nuclei (PNCN) defects in the stage 14 egg chambers. Overall, these findings reveal a novel role for Srp in regulating PCD in *Drosophila* ovaries and such a role may be conserved.

1040T **Interactions between *akirin* and *simjoang* during cardiac development** Armeta Hadjimirzaei[1], Scott J Nowak[2] [1]Molecular and Cellular Biology, KSU, [2]Molecular and Cellular Biology, Kennesaw State University

One of the most fundamental organs to form during the earliest stages of development is the heart. In the fruit fly (*Drosophila melanogaster*), many genes and proteins work together for the formation of a fully functioning heart. The conserved nuclear transcription cofactor Akirin has been identified as a key regulator of both the skeletal and cardiac myogenesis programs in *Drosophila.* The current model for Akirin function holds that this regulation of myogenesis by Akirin occurs through interactions with chromatin remodeling complex activity. Earlier work in the Nowak lab found that *akirin* works with the NuRD/CHD chromatin remodeling complex to facilitate proper expression of the cardiac gene program. For this study, we focused upon interactions between *akirin* and *simjoang* (*simj*), a key component of the NuRD/CHD complex. Using live confocal imaging, we recorded heartbeat patterns in a variety of combinations of single and double heterozygous embryos bearing mutations in *akirin* and *simj*. Our data indicates that interactions between these two loci are critical for the cardiac and skeletal muscle patterning process, strongly suggesting that NuRD/CHD activity through interaction with Akirin is a key regulator of these developmental programs.

1041T ***eEF1a2* has essential roles for a cytoskeleton homeostasis in aged fly muscles, which is independent from protein translations** Hidetaka Katow, Hyung Don Ryoo Cell Biology, New York University

The translation elongation factor eEF1α (eukaryotic elongation factor 1α) delivers aminoacyl-tRNAs to ribosomes during mRNA translation elongation. Many metazoan species have at least two eEF1α paralogs. In mammals and *Drosophila*, eEF1α1 is expressed ubiquitously, while eEF1α2 exhibits tissue-specific expression in neurons and muscle cells. Although eEF1α2 has been reported to be associated with certain neuronal diseases in humans and neuro degenerations in neuro muscular junctions, its role in muscle tissues remains poorly understood due to the short lifespan of mutant mice.

Therefore, we aimed to generate a novel eEF1α2 mutant using CRISPR/Cas9 gene editing, resulting in two loss-of-function mutants that remained viable for over a month.

In this study, we discovered that eEF1α2 plays an essential role in the cytoskeleton homeostasis of *Drosophila* indirect flight muscles. Specifically, eEF1α2 mutants exhibited thinner myofibrils in adult indirect flight muscles. With aging, these muscles showed uneven distribution of actin and myosin. Moreover, the mutant muscles developed actin- and myosin-rich structures ensheathing the trachea at >30 days after eclosion. These phenotypes were not rescued by eEF1α1, indicating that eEF1α2 functions in muscle cannot be substituted by its paralog. Notably, the mutant larval muscles did not exhibit adult-like phenotypes.

1042T **Investigating the nuclear requirement for Tnpo-SR in *Drosophila* germline stem cell self-renewal** Lovens Paul, Amanda Powell, Elizabeth Ables Biology, East Carolina University

Stem cells, with their unique capacity for self-renewal and differentiation into diverse cell types, are veritable engines driving tissue homeostasis, repair, and regeneration. Stem cell self-renewal has been extensively studied in *Drosophila* germline stem cells (GSCs), where stem cell function lays the foundation for the perpetuation of the germ cell lineage and the continuity of

life across generations. The intricate balance between self-renewal and differentiation that GSCs maintain is tightly regulated by a cadre of molecular actors, including the beta-importin Transportin-Serine/Arginine Rich (Tnpo-SR). Beta-importins are thought to promote nucleocytoplasmic trafficking; however, this class of proteins is also critical for moving mitotic spindle-associated cargo within the nucleus after nuclear envelope breakdown to promote mitotic spindle assembly. Here, we asked whether Tnpo-SR functions primarily in the GSC nucleus, cytoplasm, or both. First, we used a germline-enabled Tnpo-SR::HA transgene to track its intracellular localization across the phases of the cell cycle. We found that Tnpo-SR localizes to the nuclear membrane, nucleus, and cytoplasm, but moves to the chromatin during mitosis. Then, we aligned Tnpo-SR with human Tnpo3 and identified specific amino acids predicted to impact Tnpo-SR localization and cargo binding. Using this information, we designed a variety of germline-enabled UAS-containing transgenes that should alter Tnpo-SR localization or cargo binding in GSCs. These tools will allow us to pinpoint the exact cellular sites where Tnpo-SR is required for GSC regulation. Identifying the specific cargo molecules transported by Tnpo-SR is essential for comprehending the molecular signaling pathways and interactions that drive GSC fate decisions. Bridging these gaps in knowledge not only enhances our understanding of *Drosophila* GSC biology but also has broader implications for potential applications in human biomedical research, given the conserved nature of Tnpo-SR mechanisms across species.

1043T **Satellite DNA Regulation and the Mechanism of Segregation Distorter** Xiaolu Wei[1], Logan Edvalson[2], Amanda Larracuente[2] [1]Biomedical Genetics, University of Rochester, [2]Biology, University of Rochester

Meiotic drivers create genetic conflict by biasing their transmission to subsequent generations at a cost to the host organism. These conflicts arise in many taxa and may influence genome evolution, but little is known about how they exploit gametogenesis. Our lab studies the mechanism of the autosomal driver, Segregation Distorter (SD), in Drosophila melanogaster. SD is a sperm killer: SD/+ heterozygous males transmit SD to 95% of their offspring, whereas SD/+ females transmit SD fairly, to 50% of offspring. SD causes a chromatin condensation defect in wild type chromosomes during spermatogenesis through an unknown mechanism. The drive target is a large block of tandem satellite DNA repeats called Responder (Rsp). The role of the Rsp satellite in SD drive is unknown. Our earlier work showed that Rsp behaves as a dual-stranded piRNA cluster and is expressed early in spermatogenesis. Because piRNAs play a role in establishing silent chromatin, we hypothesized that the Rsp-derived piRNAs might be involved in drive. We combine cytological, genetic, and genomic approaches to test this hypothesis. We found that Rsp-derived transcripts, and particularly piRNAs, are reduced in SD/+ heterozygote testes. The abundance of Rsp transcripts correlates with the strength of drive. These data suggest that the mechanism of SD may involve dysregulation of Rsp RNA transcripts. Further study of SD may provide insights into drive mechanisms and how satellite DNAs are regulated during spermatogenesis.

1044T **Growth-critical Adipokines and their Role in Adipocyte to Ovary Signaling in *Drosophila melanogaster*** Chad Simmons, Tancia Bradshaw, Alissa R Armstrong Biological Sciences, University of South Carolina

Obesity, a longstanding health concern, contributes to the risk for several pathophysiologies including type two diabetes, cancer, and reproductive issues. In addition to adipose tissue accumulation, obesity disrupts the endocrine function of adipocytes, leading to altered inter-organ communication. Using *Drosophila melanogaster* as a model organism, our goal is to identify the fat-derived molecules that relay nutritional information to other organs. Recently, several adipokines have been shown to be critical for tissue growth during larval development. We set out to determine if these adipokines are also utilized for inter-organ signaling in adults. Since we know that nutrient sensing by the *Drosophila* fat body influences oogenesis, we focus on how these adipokines with known roles in larval development may mediate fat-to-ovary communication in adults.

Our study focuses on upd2, egr, ccha1, ccha2, gbp3 and sun; a group of adipokines that are critical to larval development, nutrient-availability signaling, and growth. Utilizing the Gal80^{ts}/Gal4/UAS system we knocked down individual adipokines in adult adipocytes. We have examined ovulation, germline stem cell (GSC) maintenance, and progression through vitellogenesis using whole mount immunofluorescence. Triglyceride content and adipocyte size were examined to determine the effects on the fat body. Knockdown of upd2 and egr in adult adipocytes does not affect GSC or cap cell numbers in the germaria; but disrupted vitellogenesis. Gbp3 knockdown in adipocytes also did not affect GSCs or cap cell counts, but did alter the adipocyte morphology, with fat bodies having lower triglyceride amounts and smaller cell size. In future experiments, we will examine how fecundity is affected by adipokine knockdown. Additionally, we will determine how dietary changes influence adipokine production and if they are relevant for maintaining adipokine to ovary signaling.

1045T **Investigating the role of *SRPK* in *Drosophila* germline stem cells** William G Outlaw, Amanda Powell, Elizabeth Ables Biology, East Carolina University

Oogenesis is defined as the creation of female gametes (oocytes). In *Drosophila*, this process begins in the germarium, where the germline stem cells (GSCs) are housed. Each GSC divides asymmetrically, producing a cystoblast that divides mitotically divides another four times. A single oocyte differentiated from within the resulting 16-cell cyst. Previous studies demonstrated

that the b-importin, *Transportin-Serine/Arginine rich* (*Tnpo-SR*), is necessary for GSC self-renewal and oocyte differentiation. Tnpo-SR is thought to bind cargo proteins and facilitate their movement across nuclear pores. In some cells, cargo recognition by Tnpo-SR requires the cargo to be phosphorylated prior to binding. *Serine/Arginine Protein Kinase* (SRPK) phosphorylates serine-arginine-rich proteins (SR proteins) in the cytoplasm to enable their intracellular transport into cell nuclei. Although roles for SRPK in meiotic spindle formation have previously been described, it has not been studied for a potential role in GSC self-renewal. We hypothesize that SRPK phosphorylates specific cargo, such as microtubule binding proteins, to promote the successful asymmetric division of GSCs. In support of this hypothesis, we find that SRPK is endogenously expressed in GSCs and localizes to the cytoplasm. We are currently testing whether GSC maintenance is impacted by depletion of *SRPK* specifically in germ cells. These localization and loss-of-function experiments will help to explain the potential molecular mechanisms by which Tnpo-SR promotes GSC self-renewal.

1046T **The Drosophila TENT5 homology is required for spermatid individualization** Abdulqater Al-nouman[1], Kyle Helms[2], Jennifer Curtiss[1] [1]Biology, New Mexico State University, [2]Neurology, Columbia University

The transcription factor Eyeless induces ectopic eyes when mis-expressed. We mis-expressed Eyeless in the wing and performed transcriptomics to identify upregulated eye specific transcripts. Another lab generated data from an Eyeless CHiP-seq experiment. The *CG46385* gene appeared in both data sets, suggesting that *CG46385* is a direct transcriptional target of Eyeless and plays a role in eye development. *CG46385* is predicted to encode a non-canonical polyA polymerase. Vertebrate orthologs of *CG46385* have polyA polymerase activity and physically interact with Smad transcription factors; some have been implicated in human male fertility. We used the CRISPR-Cas9 system to generate mutations in *CG46385*. None of these mutations have obvious effects on eye development; however, a mutation in the CG46385 catalytic domain renders homozygous males sterile. Individualization occurs after terminal differentiation during spermatogenesis in *Drosophila.* Before individualization, 64 elongated spermatids form a syncytium. During individualization, 64 actin cones assemble around each spermatid nucleus to form an individualization complex (IC). The IC forms a cystic bulge that travels down the axonemes of each spermatid laying down plasma membrane and stripping away unneeded organelles and cytoplasm until it reaches the distal end of the cyst. The IC pinches off into a waste bag and components are degraded via a non-apoptotic caspase cascade. Cleaved caspase-3 is active initially around the spermatid nuclei but is then restricted to the cystic bulge and the waste bags. As the IC travels down the spermatids active caspases are removed from the individualized regions. Immunofluorescence for actin and caspase-3 shows that *CG46385*$^{2\text{-}83}$ homozygous mutants have defects in spermatid individualization, as waste bags are absent and the actin cones that travel down the axonemes display a scattered configuration. Cleaved caspase-3 expression is also ubiquitous in cysts, rather than being restricted to the cystic bulge. *In situ* hybridization reveals that *CG46385* transcripts are present in elongating spermatids and localize in a “comet” expression pattern. Cup and comet genes are post-mitotically transcribed and localize to the distal ends of the elongating spermatids. Mutants for some of these cup and comet genes result in failure of spermatids to individualize. Together this work demonstrates that *CG46385* is essential during *Drosophila* spermatogenesis for spermatid individualization.

1047T **Exercise effects on the female germline stem cell lineage in *Drosophila melanogaster*** Sofia Bachmann[1,2], Emily M Wessel[1,2], Daniela Drummond-Barbosa[2,3] [1]University of Wisconsin Madison, [2]Morgidge Institute for Research, [3]Genetics, University of Wisconsin Madison

Exercise exerts a profound influence on human physiology and is popularly known as a ‘magic bullet’ for overall health. However, it remains unclear how regular physical activity affects adult stem cell lineages, which are pivotal for maintaining the structure and function of various tissues and organs. The *Drosophila melanogaster* female germline stem cell lineage has well-characterized responses to dietary and physiological factors, and *Drosophila* is also amenable to the study of exercise physiology. As a first step to test how germline stem cells are affected by exercise, we designed an improved exercise machine based on previous models that capitalize on the natural negative geotaxis response of *Drosophila*. To validate the efficacy of our new exercise machine, we analyzed *Drosophila* activity levels, fat stores, and muscle physiology of exercised compared to non-exercised control females. In the future, we plan to use our optimal exercise regimen to uncover if and how germline stem cells and their differentiating daughter cells respond to regular exercise.

1048T **CG14767: a novel regulator of the Hippo/Yki tumor suppressor pathway** Swastik Mukherjee, Alexey Veraksa Biology, University of Massachusetts at Boston

The Hippo/Yorkie (Yki) tumor suppressor pathway is an evolutionarily conserved system in metazoans that controls cell proliferation, differentiation, and cell death. The Hippo pathway is regulated by endosomal trafficking; however, the underlying mechanism is not well understood. In a screen for *Drosophila* Yki-interacting proteins, we identified a novel interactor, CG14767, that may be involved in endocytic regulation of Yki function. The mammalian orthologs of CG14767 belong to the lysosomal-associated protein transmembrane (LAPTM) family. They are localized in late endosomes and play multiple roles in

mammalian cells. However, the function of *Drosophila* CG14767 is unknown. The goal of this work is to uncover the molecular mechanisms by which CG14767 controls Yki activity at the level of endosomal trafficking. Using protein-protein binding assays, we have shown that the binding between CG14767 and Yki is mediated via the PPxY motif/WW domain interaction. Genetic tests have revealed that CG14767 is both required and sufficient for limiting the growth of adult *Drosophila* wings and suppressing Yki-induced overgrowth in adult *Drosophila* eyes. It is also required to limit the Yki-induced overgrowth in third instar larval wing discs. CG14767 and Yki colocalize in cultured *Drosophila* S2 cells, and further colocalization studies have revealed the involvement of CG14767 in the endolysosomal trafficking, where it likely recruits Yki. We hypothesize that CG14767 inhibits Yki activity either by sending it for degradation in the lysosome or by cytoplasmic retention. We are addressing this hypothesis by investigating the cellular and molecular mechanisms of CG14767 function using a combination of genetics and cell biological approaches. This research will expand our knowledge of the mechanisms of Yki regulation and will provide further insights into the regulation of Hippo signaling by the endolysosomal system.

1049T **Roles of the insulin producing cells and the fat body in nutrient-dependent neuroblast reactivation from quiescence** Susan E. Doyle, Sarah E. Siegrist Biology, University of Virginia

Precise control of cell growth and proliferation is crucial for normal tissue development and function and involves the integration of intrinsic cellular programs with extrinsic cues. In *Drosophila*, proliferating neural stem cells, called neuroblasts, enter a period of mitotic quiescence at the end of embryonic development and reactivate in response to extrinsic nutrient cues as larvae hatch and begin feeding. Both the insulin producing cells of the brain (IPCs) and the fat body, a key nutrient sensing organ, have been implicated in nutrient-dependent activation of the PI3K growth pathway and subsequent neuroblast reactivation. In the case of the IPCs this occurs through direct release of insulin-like proteins, while the fat body has been proposed to indirectly promote insulin release. Here, we examine the relative roles of the fat body and the insulin producing cells in nutrient-dependent neuroblast reactivation from quiescence. We describe the time course of fat body development during the first 24 hours of larval life and compare this to the time course of neuroblast reactivation. Using animals in which either the fat body or the IPCs have been ablated, we compare the effects of loss of each of these cell types on the timing of neuroblast reactivation. Lastly, we use cultured larval brain explants to explore a previous report that the fat body releases a mitogen capable of promoting neuroblast reactivation from quiescence. Our results suggest that the insulin producing cells promote neuroblast reactivation to a greater extent than the fat body; however, coordination of signaling between both tissues is required to ultimately achieve proper timing of nutrient-dependent neural stem cell growth and proliferation.

1050T **Zrf1 regulates the genome integrity of adult intestinal stem cells crucial for midgut regeneration** Joshua SS Li[1], Ying Liu[1], Jun Xu[2], Weihang Chen[1], Baolong Xia[1], Ah-Ram Kim[1], Yanhui Hu[1], Richard Binari[1], John M Asara[1], Norbert Perrimon[1] [1]Harvard Medical School, [2]Chinese Academy of Sciences

The *Drosophila* midgut has emerged as a powerful model to identify genes that regulate intestinal stem cell (ISC) proliferation. Previously, our lab used both RNA Pol II occupancy profiling and single cell transcriptomics to uncover genes distinctly expressed in specific cell types in the fly midgut. Building upon this, we sought to assess the function of transcription factors enriched in midgut progenitors. In an RNAi screen targeting DNA binding proteins, we identified *Zrf1* as a regulator of ISC proliferation. During homeostasis or in response to damage, Zrf1 loss-of-function decreases ISC proliferation independent of cell death. Epistasis analyses find that Zrf1 integrates multiple signaling pathways via Myc. Co-IP experiments find that Zrf1 physically associates with components of the RNA induced silencing complex. Interestingly, Zrf1 is not required for RNA interference. Instead, Zrf1 dominantly modifies position effect variegation and retrotransposon expression indicative of a role in chromatin silencing. Our findings emphasize the importance of maintaining the genome integrity of adult ISCs crucial for midgut regeneration.

1051T **Molecular insights from RNA-Seq analyses on the mechanisms governing shape and cohesion of collectively migrating border cells** Rehan Khan[1], Pralay Majumder[2], Jocelyn McDonald[1] [1]Division of Biology, Kansas State University, [2]Life Sciences, Presidency University, India

During development and in diseases such as cancer, many cells move as collectives, but the mechanisms are poorly understood. *Drosophila* border cells represent an excellent model for investigating how cell collectives migrate in vivo. In the ovary, 6-10 follicle cells form the border cell cluster, which moves collectively between large nurse cells towards the oocyte. Border cells need to stay cohesive while navigating this complex tissue microenvironment. We previously showed that Protein phosphatase 1 (Pp1) promotes border cell migration, cell shapes, and cluster cohesion. Loss of Pp1 activity disrupts actomyosin contractility and cell-cell adhesion, causing cluster disintegration into individual round cells. We also found that nurse cells, and the surrounding tissue, impose compressive forces onto border cells. Overexpression of RhoGEF2 in nurse cells led to RhoA GTPase-dependent activation of non-muscle myosin II (Myo-II), contraction of nurse cells, and compression of border cells. Here, we report our investigation to understand the molecular mechanisms of Pp1-mediated cluster cohesion and border

cell response to mechanical forces. We performed two RNA sequencing experiments using Pp1-inhibited border cells (cluster cohesion) and wild-type border cells from egg chambers with RhoGEF2-expressing nurse cells (mechanical compression). The RNA-seq results were compared to control unmanipulated border cells. Our subsequent analysis identified 160 differentially expressed genes upon Pp1 inhibition, with significant enrichment of gene ontology (GO) terms related to cell-cell adhesion. We found 1153 differentially expressed genes in border cells compressed by RhoGEF2-expressing nurse cells, with significant enrichment of GO terms related to DNA replication, cell division, and protein targeting to the ER. These analyses thus reveal new insights into how border cells and other cell collectives stay cohesive and respond to physical constraints in the tissue microenvironment.

1052T **The conserved RNA binding protein Orb2 regulates cell-type-specific responses to rare codon enriched transcripts within two distinct stem cell lineages** Rebeccah Stewart[1], Scott Allen[2], Pelin Volkan[3], Don Fox[3] [1]Pharmacology and Cancer Biology, Duke University, [2]University of North Carolina at Chapel Hill, [3]Duke University

Differences in gene/protein expression are foundational to differences in cell identity, such as between undifferentiated stem cells and their differentiated progeny. One process that is understudied for its effect on gene/protein expression during differentiation is codon bias. Codon bias occurs when certain codons are rarely used compared to their synonymous counterparts and is present throughout all forms of life. To begin to understand how codon bias impacts development and differentiation, we completed an animal wide, reporter-based screen in the model organism *Drosophila melanogaster*. This screen revealed cell type- and tissue-specific responses to codon bias. Specifically, we found the testis and brain are unique in their ability to express protein derived from rare-codon enriched reporters. In these tissues, we find robust protein expression from reporters enriched in rarely used codons, whereas the other tissues in the fly do not express such reporters. Upon further investigation in the brain, we found differential regulation of protein expression from rare-codon enriched transcripts within stem cell lineages. Neural stem cells are unable to express reporters or endogenous genes enriched in rare codons, while differentiated neurons do so robustly. To uncover molecular regulators that enable neurons to specifically express rare codons, we conducted a targeted genetic screen of 54 candidate regulators. This screen identified the conserved cytoplasmic polyadenylation element binding (CPEB) protein Orb2 and its translational coregulator CG4612 as specific regulators of rare-codon enriched transcript translation in neurons. Using both RNA FISH and RNA-seq we then identified endogenously expressed rare-codon-enriched mRNAs regulated by Orb2, including the G-protein coupled receptor mGluR. These rare-codon enriched mRNAs are linked to Orb2's function in long term memory in specific neuronal cell types. In the testes, there is increased translation of a rare-codon enriched reporter and endogenous rare-codon enriched genes during spermatid differentiation. We have also found a role for Orb2 in increasing translation of the rare-codon enriched reporter in spermatids. Orb2 has an already described role in localizing mRNAs to the axoneme of differentiating spermatids with a measurable impact on fertility. In both the brain and testes, we hypothesize rare codons are needed for gene expression regulation mediated by Orb2 during differentiation. Using our *Drosophila* model, we have uncovered critical regulation of rare-codon enriched transcript expression driven by Orb2. Our findings reveal dynamic central dogma regulation within defined stem cell lineages that drives cellular differentiation and function.

1053T **Function of *CG11180/Chigno* in adult and developing *Drosophila* ovaries** Cheng Yang[1], Pedro Massa[1,2], Natalie Luffman[1,3], Matthew J Wawersik[1], Oliver P Kerscher[1] [1]Biology, College of William & Mary, [2]Division of Pre-Clinical Innovation, National Center for Advancing Translational Science, National Institutes of Health, [3]Human & Molecular Genetics Department, Virginia Commonwealth University, School of Medicine

CG11180 or *Chigno* is a novel protein identified in *Drosophila melanogaster* which exhibits functions in regulating cell differentiation and maintenance in reproductive systems. Our lab has found that *Chigno* is expressed in germline and somatic cells within both testes and ovaries. In addition, somatic inhibition of *Chigno* in testes and ovaries disrupts stem cell regulation and causes infertility. Here we investigate the reasons for these effects, specifically in adult and developing fly ovaries. Data examining *Chigno* expression pattern in adult ovaries is presented, focusing on the germarium which houses two stem cell niches. *Chigno* expression during ovary development is also shown. Furthermore, we explored the impacts of *Chigno* inhibition on somatic and germ cell morphology, behavior, and fate. Our results suggest that *Chigno* is required in adult ovarian follicle stem cells (FSCs), FSC progeny, and in the germline. Somatic *Chigno* inhibition causes accumulating FSC-like cells and pre-follicle cells (pFCs), suggesting that *Chigno* normally acts to promote FSC and pFC proliferation and/or differentiation. *Chigno* may also have important roles in maintaining the extended morphology of escort cells (ECs) that regulate differentiation of germline stem cells (GSCs) and their progeny in the germaria. Moreover, *Chigno* inhibition in the germline cause germ cell loss and defects in development of the somatic ovary, suggesting that *Chigno* is essential for germ cell viability during development, which, in turn, ensures normal development of adjacent somatic cells. Additional data examining the potential mechanisms by which these defects manifest after somatic or germline *Chigno* inhibition is also presented. The human homolog of *Chigno*, *PINX-1*, is localized to the nucleolus and functions as a telomerase inhibitor and tumor suppressor in many vital tissues and organs including liver, lungs, mammary, and reproductive tissues. Its reduced expression in ovarian

epithelial cells is also associated with increased ovarian cancer severity and increased tumor metastasis. As a result, our analysis of *Chigno* function has implications for our understanding of *PINX-1*'s role in cancer, infertility, and stem cell regulation.

1054T **Organization of apical-cortical Actin in the primary pigment cells of the *Drosophila* pupal eye** Abhi Bhattarai, Emily W McGhie, Joshua Woo, Ruth I Johnson Biology, Wesleyan University

The pseudostratified epithelium of the *Drosophila* pupal eye consists of around 750 ommatidia. Each ommatidium is composed of four central cone cells and two primary pigment epithelial cells that sit on top and completely surround a cluster of eight photoreceptors. Secondary and tertiary pigment epithelial cells form a lattice that separates each ommatidium. We find that the primary pigment cells, in particular, become characterized by the dense accumulation of apical-cortical actin filaments, that become radially organized. We name these Apical Radial Actin Filaments (ARAFs). The ARAFs appear to traverse the entire width of primary pigment cells, and non-muscle myosin II (NMII) accumulates along their length, suggesting that the ARAFs are contractile. We have found that two formins, Diaphanous (Dia), and Dishevelled Associated Activator of Morphogenesis (DAAM) are mainly responsible for the nucleation of the ARAFs. Further, the *Drosophila* Villin-family protein Quail (Qua) accumulates in primary pigment cells and is essential for ARAF integrity, suggesting that Qua bundles and anchors ARAFs. A second bundling protein, α-Actinin (ACTN), is also crucial for ARAF structure. Our 3D imaging reveals that ARAFs are required to refine the shape of maturing primary pigment cells.

1055T **The serine-like protease *masquerade* (*mas*) plays an important role in tracheal tube formation** Victoria Kurdyumov, Alexa Oldenkamp, Afshan Ismat Biology, University of St. Thomas

The embryonic trachea is a convoluted series of epithelial tubes that provide oxygen to all cells and tissues in the embryo. Proper formation of these tubes involves several distinct cellular processes, including invagination, collective migration, and cell intercalation. *Masquerade* (*mas*) is a serine-like protease that is expressed in the embryonic trachea. In the absence of *mas*, we found individual tracheal metameres had missing dorsal trunk or dorsal branches while the rest of the trachea formed normally. Moreover, we found that, when the lumen was missing, tracheal nuclei were not present. Using btl-GAL4::UAS-myrGFP, which marks the entire cell membranes of tracheal cells, we found misshapen tracheal cells and uneven dorsal trunks. Currently, we are looking at cell-cell junction markers. We are also in the process of generating over-expression constructs of *mas* to see what happens in the trachea with too much Mas present. We also are in the process of looking at whether there is a link between mas and FGF signaling in the trachea. Overall, this work will be instrumental to our understanding of tracheal tube formation.

1056T **Bourbon and Mycbp function with Otu to promote the expression of Sxl in the Drosophila female germline** Marianne Mercer, Michael Buszczak Molecular Biology, UT Southwestern

In Drosophila ovaries, germ cells differentiate through several stages of cyst development before entering meiosis. This early differentiation program depends on both the stepwise deployment of specific mRNA translation regulatory mechanisms and on maintenance of germline sexual identity. The study of female sterile mutations that result in formation of germ cell tumors has been invaluable in identifying the molecular mechanisms that control these developmental events. We identified a germ cell enriched gene *bourbon* (*bbn*), null mutants of which cause the formation of cystic germ cell tumors. Loss of *bbn* causes inappropriate and overlapping expression of Nanos (Nos) and Bag-of-marbles (Bam). To attempt to better understand the function of Bbn, we performed proteomic analysis and found Bbn forms a complex with the Drosophila ortholog of cMyc binding protein (Mycbp) and Ovarian tumor (Otu), a protein previously linked with regulation of the sex determination factor S*ex lethal* (S*xl*). Loss of *Mycbp* results in the formation of cystic germ cell tumors, mimicking the differentiation defects observed in *Sxl*, *otu*, and *bbn* mutants. Bbn promotes the stability of Otu and fosters interactions between Otu and Mycbp. We find that germ cells from *bbn* mutants display a loss of Sxl expression specifically in the germline. Strikingly, transgenic expression of *Sxl* rescues the *bbn* sterile phenotype, indicating one of the primary functions of this complex is to promote cytoplasmic Sxl expression. Lastly, we find that the human ortholog of Otu, OTUD4, and Mycbp also physically interact. Together these data indicate that Bbn, Otu and Mycbp form a conserved complex that promotes the differentiation of Drosophila germ cells.

1057T **Detailing the functions of Cytokine/JAK/STAT signaling during *Drosophila* midgut regeneration** Xiaoyu Kang[1], Bruce Edgar[2] [1]Oncological Sciences, Huntsman Cancer Institute, University of Utah, [2]Oncological Sciences, Huntsman Cancer institute, University of Utah

Understanding cytokine/JAK/STAT signaling is crucial, as it plays central roles in immune and inflammatory responses, regeneration, wound healing, and human diseases such as auto-immune disorders, inflammatory bowel diseases (IBD), and cancers. While the human inflammatory response is intricate, involving numerous cell types and cytokines, the *Drosophila* system offers a simpler model, with only three cytokines, Unpaired 1, 2, 3 and a single gene encoding their

receptor, Domeless (Dome). It also has only one Janus Kinase (JAK; Hopscotch (hop)), and one known STAT transcription factor, STAT92E. Our previous research unveiled that *Drosophila* cytokines Unpaireds (Upds), functioning akin to human IL-6, are massively induced in response to gut epithelial damage. They, in turn, trigger gut epithelial regeneration through Jak/Stat signaling. However, it is still not known what specific roles cytokine signaling plays in the different gut cell types, or what the target genes of this pathway are in each cell type. To elucidate how Upds induce gene expression during stress-dependent gut regeneration, we plan to map the gene targets of the cytokine effector, STAT92E, in the different midgut cell types through the regeneration process by combining CUT&TAG (to map the DNA binding sites of STAT92E) with RNA-seq and multiplexed FISH (to catalog the transcriptional output of STAT92E). Following this mapping survey, our study will investigate selected STAT target genes to determine their roles in intestinal stem cell (ISC) activation and their functions in the other midgut cell types. Specifically, we aim to address the following questions: 1) How does cytokine/Jak/Stat modulate stem cell transcription to promote proliferation? 2) What is its specific function in differentiation in the ISC lineage? 3) How does it interact the EGFR signaling pathway? We believe our findings will offer a valuable model for epithelial regeneration with broader implications. For example, understanding the mechanism of how IBD promotes tumorigenesis.

1058T **Regulating Hippo signaling, planar cell polarity, and metabolism through the Fat intracellular domain** Cole Julick[1], Nattapon Thanintorn[1], Yi Qu[2], Yonit Tsatskis[3], Helen McNeill[1] [1]Developmental Biology, Washington University School of Medicine in St. Louis, [2]Lunenfeld-Tanenbaum Research Institute, [3]The Hospital for Sick Children

Development requires sophisticated signal transduction systems to coordinate organ and tissue growth, metabolism, and cellular orientation and patterning. Fat (Ft) and Dachsous (Ds) are a pair of large, evolutionarily conserved cell adhesion molecules that act as a ligand-receptor system which regulates and coordinates these processes. In *Drosophila*, Ft functions at the cell surface where it preferentially binds to Ds in a heterophilic fashion. The interaction between these two cadherins initiates bidirectional signaling mediated by their intercellular domains (ICD) to regulate growth by influencing Hippo signaling and morphogenesis by regulating Planar Cell Polarity (PCP). The ICD of Ft contains multiple mitochondrial targeting signals that, when cleaved, release a fragment that is imported into mitochondria where it binds to and stabilizes Complex I suggesting mitochondrial signaling may also act to direct PCP. The ICD of Ft consists of 6 regions that are conserved between *Drosophila* to human. However, the complete function of these conserved regions isn't fully understood. We take advantage of the simplicity and accessibility of *Drosophila* larval wing imaginal discs to identify genes that play crucial roles in development. To understand the mechanisms by which Fat-ICD regulates Hippo signaling and PCP, we utilized CRISPR to make targeted deletions of the 6 conserved blocks of amino acid. We characterized each CRISPR line via RNA sequencing and were able to identify different gene expression profiles for the various lines. This allows for thorough examination of the role each conserved region plays in growth, development, and patterning. We identify mutations within the conserved regions in the Ft-ICD that impair Fat-Hippo signaling and PCP. Specifically, we find variation in wing size, shape, and symmetry along with PCP and gene expression. Our research provides further insight into the endogenous role of the conserved regions within the ICD.

1059T **Synergistic activation by Glass and Pointed promotes neuronal identity in the *Drosophila* eye** Hongsu Wang[1], Komal Kumar Bollepogu Raja[2], Kelvin Yeung[2], Carolyn Morrison[1], Antonia Terrizzano[1], Celine Tran[1], Alireza Khodadadi-Jamayran[3], Cornelia Fritsch[4], Simon Sprecher[4], Graeme Mardon[2,5], Jessica E Treisman[1] [1]Cell Biology, NYU Grossman School of Medicine, [2]Pathology and Immunology, Baylor College of Medicine, [3]Applied Bioinformatics Laboratories, NYU Grossman School of Medicine, [4]Universite de Fribourg, [5]Molecular and Human Genetics, Baylor College of Medicine

Progenitor cells can be directed to differentiate into distinct cell fates by a combination of extrinsic signals and intrinsic transcription factors. In the eye imaginal disc, the Glass (Gl) transcription factor is expressed in photoreceptors as well as non-neuronal cone and pigment cells, and acts autonomously in each cell type to promote its normal differentiation. Single-cell RNA-Seq analysis of *gl* mutant eye discs revealed that the differentiation of all cell types is disrupted. The first photoreceptor to form, R8, is the least affected, but few of the later-differentiating cell types are observed. R8 induces other photoreceptors to differentiate by activating Epidermal Growth Factor Receptor (EGFR) signaling, which is transduced by the transcription factor Pointed (Pnt). To understand how Gl and EGFR signaling interact in the absence of upstream retinal determination factors, we ectopically expressed Gl and/or the EGFR pathway activator Ras^{V12} in the wing disc. The combination of both factors, but neither alone, induced the expression of many genes associated with neuronal differentiation. This synergy required *pnt*, indicating that it occurs at the transcriptional level. We used targeted DamID to profile Gl and Pnt binding sites in eye disc cells at the onset of differentiation and in committed photoreceptors. We found that many enhancer regions were bound by both transcription factors in one or both conditions, including enhancers for some genes that were synergistically induced in the wing disc. Motif analysis suggested that each factor is able to recognize less optimal binding sites in co-bound regions, potentially due to cooperative binding. The genes directly bound and co-regulated by Gl and Pnt include both transcription factors and downstream effector genes, suggesting that Pnt and Gl promote neuronal identity through a multi-layered transcriptional network. We also found that Gl can directly activate the transcription of genes specific to non-neuronal retinal cells, independently of Pnt. These results shed light on the interactions between signal-regulated and intrinsic

transcription factors that enable a pool of equivalent progenitor cells to differentiate into multiple cell types.

1060T **Investigating the role of the Drosophila PI 4-kinase Four wheel drive during spermatocyte cytokinesis** Catherine Q.F. Zhang[1,2], Anastasia Fexa[1,2], Yonit Tsatskis[2], Julie A. Brill[1,2] [1]Molecular Genetics, University of Toronto, [2]Cell Biology Program, The Hospital for Sick Children

During sperm development, successful meiotic cytokinesis is essential to prevent multinucleate cells that can lead to infertility. Using the model of Drosophila melanogaster spermatogenesis, the phosphatidylinositol (PI) 4-kinase III β (PI4KIIIβ) "Four wheel drive" (Fwd) was identified as essential for meiotic cytokinesis. However, the mechanistic role of Fwd's lipid product, PI4P, during meiotic cytokinesis is not fully understood. In addition, the regulators that control the localization of Fwd at the Golgi during spermatogenesis are unknown. Using high-resolution time-lapse live imaging, markers to visualize the centralspindlin complex, spindle microtubules, actomyosin ring, plasma membrane, and membrane trafficking will be used to pinpoint the primary defect in *fwd* mutants during meiotic cytokinesis. To identify regulators of Fwd, analysis of putative interactors of Fwd by co-immunoprecipitation revealed a conserved interaction between Fwd and the Drosophila orthologs of mammalian proteins ARMH3 (CG8379) and ACBD3 (CG14232), which regulate PI4KIIIβ. Both proteins localized to the Golgi in Drosophila S2 cells and will be characterized as candidates that may recruit Fwd to the Golgi during spermatogenesis. In addition, novel interactors and putative regulators of Fwd will be identified using proteomics methods including AP-MS and BioID. Overall, uncovering the role of Fwd during meiotic cytokinesis will allow for insight into conserved mechanisms regulating this universal process.

1061T **Identifying polarity factors of Crumbs-induced neoplastic tissue growth** Max Shcherbina, Parama Talukder, Ulrich Tepass Cells & Systems Biology, University of Toronto

Crumbs (Crb), an apical transmembrane protein, plays a pivotal role in regulating epithelial polarity and influences tissue growth through its interactions with the Hippo and JNK pathways. Both in mammals and Drosophila, the Crb protein has been implicated in tissue growth regulation and tumour development. Notably, Crb overexpression in Drosophila imaginal disc epithelia induces neoplastic overgrowth. We are interested in exploring which factors are required for neoplastic tumourigenesis when Crb is overexpressed.

Moderate Crb overexpression activates the Hippo pathway effector, Yorkie, alongside the JNK pathway, leading to over-proliferation and neoplastic transformation of the epithelium. Surprisingly, high-level Crb overexpression switches JNK's role from promoting proliferation to inducing apoptosis, thereby reducing tumour size. Reducing JNK activity in this context seemingly restores full neoplastic growth, suggesting that high-level Crb overexpression triggers enhanced JNK activation, thus offsetting Crb's pro-proliferative effects.

Our focus has expanded beyond JNK signalling to include components of the epithelial polarity machinery in the context of Crb-induced neoplastic development. Testing Crb constructs with modified cytoplasmic domains, we discovered that removing either the C-terminal PDZ domain or the juxtamembrane FERM domain noticeably diminished the Crb-induced neoplastic phenotype. This finding emphasizes the significance of Crb's domain binding partners in facilitating Crb-induced neoplastic growth.

Building on these experiments, our current investigations are directed toward assessing the roles of known Crb binding partners and other components of the epithelial polarity machinery. We are screening their contributions to neoplastic development, alongside other key elements of the epithelial polarity complex. This approach aims to unravel the intricate network of interactions that facilitate Crb's role in epithelial tissue regulation and its potential aberration in neoplastic processes. Our goal is to investigate the precise molecular mechanisms through which these polarity factors influence Crb-mediated neoplastic transformation.

1062T **Function of the RhoGEF Cysts in imaginal disc morphogenesis and tumorigenesis** Ming Yu Cao, Milena Pellikka, Ulrich Tepass Cell and Systems Biology, University of Toronto

Rho1 is a small signalling G protein that regulates diverse cellular processes such as cytoskeletal organization, cell movements, and cell division. The key regulators of Rho1 protein activity include Guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs). In Drosophila, RhoGEFs like RhoGEF2 and RhoGEF64C , as well as RhoGAP like Cumberland GAP, have been reported to exert context-dependent control over Rho1 signaling. Nevertheless, the specific mechanisms through which GEFs and GAPs govern the spatiotemporal activity of Rho1 during wing imaginal disc morphogenesis remain largely elusive.

Our laboratory has recently characterized the RhoGEF Cysts (mammalian orthologs p114 RhoGEF,p190 RhoGEF, AKP-13,

GEF-H1) in Drosophila embryos, demonstrating its adherens junction associated activation of the Rho1–Rok–myosin II pathway, thereby promoting epithelial integrity during gastrulation. The present study aims to examine Cysts' function in the regulation of wing disc tissue growth. Our results indicate that Cysts is recruited to the apical cortex of wing disc cells by the polarity proteins Crumbs and Bazooka. Here, it plays essential roles in regulating tissue folding and cell viability as loss of Cysts led to excessive epithelium folding at the hinge region and cell death. Furthermore, when apoptosis is inhibited with the p35 baculovirus protein, Cysts knockdown led to "microtumour" formation. Those "microtumours" have upregulated Yorkie and JNK signalling and show defects in epithelial polarity. We then further analyzed Cysts role during tumour development and found that Cysts knockdown accelerated overgrowth of the wing disc and delayed larval pupariation time consistent with increased neoplastic differentiation. Moreover, the double knockdown of Cysts and RhoGEF2 led to synergistic enhancement of the growth defects. Overall, our data suggests that Cysts is an important regulator of junctional Rho1 activity in the wing disc and its cooperation with RhoGEF2 is essential for controlling Rho1 signaling during wing disc development and tumorigenesis.

1063T **Preliminary characterization of *surfeit-4* gene in *Drosophila melanogaster*** Jada Scott, Nathan Powers, Mayank Kapadia, Ajay Srivastava Biology, Western Kentucky University

Basement membranes (BM) are specialized forms of extracellular matrix that play critical roles during normal development and tumor metastasis. BM properties can be modified by several mechanisms, one of them being by changing the composition of BM itself. We identified Surfeit-4 (Surf-4) as an interacting protein in a biochemical screen for proteins that associate with BM component, Collagen-IV. To further understand the function of this gene we generated a peptide antibody, characterized it, and used it to assess the sub-cellular localization of this protein in *Drosophila* larval tissues. RNAi was used to generate phenotypes associated with this gene. Preliminary results from these endeavors will be presented and point to a localization of Surf-4 in the secretory pathway. Specific wing phenotypes were generated as well and their preliminary characterization will be presented.

1064T **Identifying GPCR genes that affect salivary gland and germ cell migration** Kailey Boyle, Samantha Snyder, Caitlin Hanlon Biology, Quinnipiac University

GPCRs are one of the largest and most widely expressed protein families, found in all manners of life from bacteria to humans. Despite GPCR's prominence throughout biology, they have been surprisingly understudied during Drosophila development. One hundred and sixteen GPCRs are encoded in the Drosophila genome, and although their sequence and many expression patterns are known, what they sense and what they do during development remains unknown. The goal of this project is to systemically investigate the role that GPCRs play in Drosophila embryonic development in the salivary gland and germ cells via an RNAi screen. This project is being completed at a primarily undergraduate institution (Quinnipiac University) by undergraduate independent study students, students participating in a CURE in a upper level Cell Physiology course, and master's level students. Thus far, 40 genes have been knocked-down in the salivary gland, and three potential hits have been identified (*CG11318*, *CG15556*, and *smog*). Twenty-five genes have been knocked-down in the germ cells, and one potential hit has been identified (*FMRFaR).* Here, we present our work knocking down *frizzled* (*fz*), methuselah-like 5 (*mthl5*), and stem cell tumor (*stet*) in the salivary gland and germ cells. We hypothesize that knockdown of *mthl5* will affect the salivary gland and that knockdown of *stet* will affect the germ cells.

1065T **Genetic and Molecular Mapping of two cell growth mutations, d.2.2 and a.2.1 in conjunction with the Fly-CURE** Jennifer Cifranic, Annie Richters, Lauren Heininger, Reese Saho, Delia Adkins, Emma Widmer, Jamie Siders School of Science, Technology, and Mathematics, Ohio Northern University

The Fly-CURE is a **c**ourse-based **u**ndergraduate **r**esearch **e**xperience implemented in undergraduate genetics courses. This CURE utilizes an EMS-based genetic screen in conjunction with the Flp/FRT system to generate hundreds of mutants with irregularities in cell division or cell growth in the eye; mutations analyzed by students are limited to the right hand arm of chromosome 2. Standard complementation analysis is then conducted to map the causative gene. The current work phenotypically characterizes and maps two of the Fly-CURE mutants: d.2.2 and a.2.1. The d.2.2 mutant displayed a standard red>white cell overgrowth phenotype in the eye, while crosses to the BDSC7896 deficiency stock failed to complement. The deletion contained on BDSC7896 maps to between nucleotide positions 20,245,186 and 20,245,490 on chromosome 2R and narrows down the candidate genes to less than 20, including plutonium (*plu*), a negative regulator of DNA replication. The a.2.1 mutant mutant displayed significant red>white cell overgrowth and scarring in the eyes. The a.2.1 mutant was crossed to 98 deficiency stocks on chromosome 2R and none of the stocks resulted in a failure to complement. It is possible the causative gene of the a.2.1 phenotype is contained within a region of 2R that is not covered by an available deficiency stock. Alternatively, it was found that some of the deficiency stocks had lost the curly wing marker on the balancer and some deficiency crosses will need to be performed again. Future work will involve crossing the d.2.2 mutant to single gene alleles of the candidate genes, including *plu*. As genetic mapping failed to identify the causative gene for a.2.1, a whole genome sequencing and Sanger sequencing of candidates in the regions not covered by deficiency stocks will be used to identify the

specific genetic lesion responsible for the cell growth phenotypes observed in the a.2.1 mutant.

1066T **Examining the function of *Dchs1* and *Dchs2* in Mammalian Eye Development** Jennysue Kasiah[1], Helen McNeill[2] [1]Washington University-St. Louis, [2]Washington University in St. Louis

Dchs1 and *Dchs2* are large protocadherins that play crucial roles in mammalian development and disease. *Dchs1* and *Dchs2* are the mammalian orthologs of *Drosophila d*achsous (*ds*). Ds, in conjunction with its binding partner fat (Ft), are regulators of tissue growth via the Hippo pathway, planar cell polarity (PCP). *Dchs1* and *Dchs2* play developmental roles in a variety of mouse tissues including kidneys, lungs, heart, brain, and the skeleton. Human diseases associated with the loss of *DCHS1* and its binding partner *FAT4*, include Hennekam and Van Maldergem syndrome.

Published RNA sequencing show *Dchs1/2* expression in a variety of retinal progenitor cells, and neurons of the retina including bipolar, amacrine, horizontal, and retinal ganglion cells (RGCs). My preliminary RNAscope and western blot data confirm that both *Dchs1* and *Dchs2* are present in the post-natal retina. To determine the role of *Dchs1/2* during retina development, I generated conditional knockouts of *Dchs1* and *Dchs2* using Tg(Rax-cre)1Zkoz (mRx-cre), which targets loss of *Dchs1/2* to the retinal pigment epithelium (RPE), retina and portions of the forebrain. Examination of Tg(Rax-cre)1Zkoz *Dchs1*$^{tm1.1IrvF/F}$ /B6 animals showed that loss of *Dchs1* did not cause any overt retinal histological changes or changes in cell differentiation/ cell number at P0, P15, or P30. Preliminary examination of Tg(Rax-cre)1Zkoz *Dchs2*$^{tm1.2FhelF/F}$/B6 animals at P17 showed an increase in the number of cells in the retina that are positive for the pan retina neuronal cell marker, PROX1, suggesting that *Dchs2*, not *Dchs1*, may play a role in retina development.

It is unknown if *Dchs1/2* serve redundant roles in the retina. Double knockouts globally and specifically in the mouse embryonic kidney demonstrated that loss of both *Dchs1* and *Dchs2* drives synergistic effects leading to greater restriction of the nephron progenitor pool than loss of either gene alone. I am generating double Tg(Rax-cre)1Zkoz *Dchs2* $^{tm1.2FHel}$ *Dchs1*$^{Ftm1.1IrvF/F}$ *tm1.2FhelF/F* /B6 mutants to determine if *Dchs1/2* have redundant roles during retinal development. I am also in the process of fully characterizing the Tg(Rax-cre)1Zkoz *Dchs2* $^{tm1.2FHel}$ /B6phenotype via immunofluorescence on whole eye cryo-sections and retina flat mounts, focusing on specific retina neuronal cell type markers as well as the pan marker PROX1.

1067T **Adams Oliver Syndrome-associated RBPJ variants act as dominant negative proteins that cause developmental defects due to compromised Notch signaling in the vasculature** Alyssa Solano[1], Kristina Preusse[2], Lisa Gutzwiller[2], Rebecca Hotz[2], Shruti Vemaraju[2], Rhett Kovall[3], Rafi Kopan[2], Brian Gebelein[2] [1]Medical Scientist Training Program, University of Cincinnati College of Medicine, [2]Developmental Biology, Cincinnati Children's Hospital Medical Center, [3]Molecular and Cellular Biosciences, University of Cincinnati College of Medicine

The rare genetic condition Adams Oliver Syndrome (AOS) is characterized by the combination of scalp lesions and transverse terminal limb defects present at birth. Previous sequencing of a large AOS patient cohort identified allelic variants in multiple components of the highly conserved Notch signaling pathway, including the ligand *DLL4*, the receptor *NOTCH1*, and the pathway's sole transcription factor *RBPJ*. Notch signaling is activated by ligand-receptor interactions that trigger the proteolytic release of the Notch Intracellular Domain (NICD), which forms a DNA binding complex with RBPJ and activates transcription. In AOS patients, six dominantly inherited autosomal missense mutations have been identified in or near the DNA-binding domain of RBPJ. The mechanism by which these allelic variants bring about AOS phenotypes is not yet known. The goal of this project is to describe the pathogenesis of autosomal dominant AOS variants in RBPJ using a combination of quantitative biochemical assays and animal models. Our current biochemical studies support the idea that AOS-associated RBPJ variants cause dominant phenotypes by weakening DNA binding but not binding to NICD. Using analogous mutations in mouse RBPJ, we investigate whether mice with AOS variants develop autosomal dominant phenotypes consistent with a dominant-negative protein function, and we test the following <u>*hypotheses:*</u> first, that AOS-associated RBPJ variants function as dominant negative proteins that compromise Notch signaling by sequestering NICD from DNA; and second, that the expression of RBPJ variants in the embryonic vasculature drives AOS pathogenesis. The completion of this research will establish the molecular mechanism by which AOS-associated RBPJ variants produce a disease phenotype and ascertain the tissue type(s) driving AOS pathogenesis and the critical temporal window(s) in which the AOS phenotype is established. This improved understanding of Notch biology will facilitate therapeutic manipulation of the Notch pathway for the treatment of AOS and other Notchopathies.

1068T ***In Silico* analysis of two novel variants in voltage gated sodium channel encoding gene SCN1A linked to Epilepsy** Chetan Ghati Kasturi Rangan[1], Yogananda S Markandeya[2], Gautham Arunachal[3], Bhupesh Mehta[4], Madhura Nimonkar[2], Prashanth Vashista[2] [1]Human Genetics, National Institute of Mental Health and Neurosciences, [2]Biophysics, Nimhans, [3]Human Genetics, Nimhans, [4]Nimhans

Dravet syndrome (DS), also known as severe myoclonic epilepsy of infancy (SMEI) is a severe intractable epileptic encephalopathy associated with early onset seizures, developmental arrest in early childhood. Seizures are often temperature sensitive. Despite the availability of several antiseizure medications, it is often a challenge to treat drug-resistant forms. The pathogenic variants in SCN1A gene, located on chromosome 2q24.3 that codes for the voltage-gated sodium channel $Na_V1.1$ leading to channel dysfunction, has been found to be a major genetic cause of this disorder. We performed whole exome sequencing for patients with DS and identified 2 missense variants NP_001159435.1:p.Ile227Thr and NP_001159435.1:p.Glu1032Lys in *SCN1A* gene. The pathogenic variant on exon 8, NM_001165963.4:c.680T>C [clinvar reported: VCV000930374.5] was de novo and leads to substitution of amino acid Isoleucine to Threonine at position 227 in the transmembrane domain of $Na_V1.1$. The variant, NM_001165963.4:c.3094G>A, on exon 19 is novel but was inherited from affected mother. It leads to the substitution of the amino acid Glutamic acid to Lysine at position 1032 in the cytoplasmic domain. To understand the association between the nature of the mutation and the resulting functional alteration, we performed extensive *in silico* analysis to understand and predict the possible pathomechanism. Multiple sequence alignment was carried out using PRALINE and Clustal Omega. In order to quantify the destabilization effects of the mutations, the difference in folding free energy change between native and mutant proteins (DDG or ddG) was calculated. The results from protein stability predictors, namely, iStable, MUpro, and I-Mutant Suite, indicated that the Ile227Thr and Glu1032Lys are leading to decrease in protein stability. The structure based prediction tool, PolyPhen 2 showed that the mutation I227T to be probably damaging with a score of 1.00 and E1032K to be benign with a score 0.21. PANTHER-PSEP, a position specific evolutionary preservation tool classified Ile227Thr to be probably damaging with a score of 0.74 and Glu1032Lys to be possibly damaging with score 0.5. Functional variant prediction was carried out using the funNCion tool which predicted Glu1032Lys to be neutral and Ile227Thr to be pathogenic with a probability of 0.67 and loss-of-function with a probability of 0.56. Results from our *in silico* analysis demonstrates the deleterious effects of the two mutations and serves as reference for planning of further *in vitro* experiments.

1069T **Spatio-Temporal Control of RNAi in Tribolium castaneum** Muhammad Salim Hakeemi University of Maryland

RNA interference (RNAi) is a natural antiviral defense mechanism in plants and animals. As a counter defense strategy, most viruses have evolved viral suppressors of RNAi (VSRs) to antagonize the RNAi pathway. Here, we utilized VSR from Cricket Paralysis virus (CrPV1A) to restrict RNAi spatiotemporally and overcome the limitation of the strong systemic RNAi response in T. castaneum. We found that ubiquitously driven VSR shows a notable rescue of sterility, which so far has hindered the analysis of RNAi mediated phenotypes of Tc-axin and Tc-dpp. Our findings demonstrate the potential use of the newly developed VSR tool on overcoming the limitation of sterility problem in T. castaneum. Moreover, our proof of concept work opens additional avenues for future applications such as separating maternal and zygotic gene function in T. castaneum.

1070T **Solubility phase transition of maternal RNAs during vertebrate oocyte-toembryo transition** Hyojeong Hwang[1], Sijie Chen[2], Meng Ma[2], Divyanshi Divyanshi[3], Hao-Chun Fan[2], Elvan Böke[4], Wenyan Mei[2], Jing Yang[2] [1]Department of Biochemistry and Biophysics, University of Pennsylvania, [2]Comparative Biosciences, University of Illinois, [3]Cell and Developmental Biology, University of Illinois, [4]4Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology

The oocyte-to-embryo transition (OET) is regulated by maternal products stored in the oocyte cytoplasm, independent of transcription. How maternal products are precisely remodeled to dictate the OET remains largely unclear. In this work, we discover the dynamic solubility phase transition of maternal RNAs during *Xenopus* OET. We have identified 863 maternal transcripts that transition from a soluble state to a detergent-insoluble one after oocyte maturation. These RNAs are enriched in the animal hemisphere and many of them encode key cell cycle regulators. In contrast, 165 transcripts, including nearly all *Xenopus* germline RNAs and some vegetally localized somatic RNAs, undergo an insoluble-to-soluble phase transition. This phenomenon is conserved in zebrafish. Our results demonstrate that the phase transition of germline RNAs influences their susceptibility to RNA degradation machinery and is mediated by the remodeling of germ plasm. This work thus identifies important remodeling mechanisms that act on RNAs to control vertebrate OET.

1071T **Regulation of meiotic cytokinesis in *S. cerevisiae*** Matt Durant, Linda Huang University of Massachusetts Boston

In *S. cerevisiae*, cells undergo sporulation in response to starvation. Sporulation is comprised of three main stages: commitment to sporulation, meiosis, and spore wall deposition. During meiosis II, each haploid nucleus is surrounded by a prospore membrane, which is synthesized *de novo*. The closure of this prospore membrane around the newly formed haploid nucleus is the meiotic cytokinesis event. The timing of prospore membrane closure is regulated by the Sps1 STE20-GCKIII kinase acting downstream of the Cdc15 Hippo-like kinase. Closure of the prospore membrane is thought to require the removal of Ssp1, a coiled-coil protein of unknown function that localizes to the leading edge of the growing prospore membrane. A complex of proteins known as the leading edge protein complex (LEP) assembles at the open end of growing prospore membranes and may keep elongating prospore membranes open as meiosis II progresses. Ssp1 is required for the other members of the LEP complex to properly assemble; removal of the LEP occurs before prospore membrane closure.

The mechanism by which the leading edge is removed from the prospore membrane is poorly understood. Interestingly, the sporulation specific MAP kinase encoded by *SMK1* localizes to the PSM in early meiosis II and becomes localized specifically to the leading edge of growing prospore membrane in late meiosis II. Smk1 localization to the LEP requires Ssp1. Our work aims to determine the mechanism by which the LEP is removed from the PSM as part of PSM closure.

1072T **Multiple Prostaglandins Play a Role in the Early Heart Development of Zebrafish (*Danio rerio*)** Jill Parsons Biology, Williams College

The patterning of heart valve precursors occurs during a critical period beginning about 24 hpf. Previous work has implicated prostaglandins in heart valve development and cardiac morphology, but these works focused on time points before and after the critical period for valve patterning. We confirm that inhibition of Cox-2, an enzyme essential for biosynthesis of prostaglandins, inhibits valve leaflet outgrowth and we narrow the period during which this effect occurs. We further investigated the role of specific prostaglandins using live imaging of beating zebrafish hearts at 96 hpf. Prostaglandins previously reported to rescue or phenocopy Cox-2 inhibition at other time points did not provoke the same effects on valve and heart morphology during this critical period. Instead, a different set of prostaglandins appear to be required for heart valve formation and heart morphology beginning at 24 hpf. Our findings highlight the dynamic use and re-use of developmental signals at different stages and in different tissue layers during the course of heart formation.

1073T **Characterizing Hybrid Cell Fate and Formation in Zebrafish Axial Mesoderm Development** Avani Modak[1], Cheng-Yi Chen[2], Jeffrey Farrell[2] [1]Unit on Cell Specification and Differentiation, National Institute of Child Health and Human Development, [2]National Institute of Child Health and Human Development

During development, genes characteristic of specific cell fates are activated in each cell. Typically, only one expression program is activated. However, recent single-cell genomic assays in zebrafish revealed that in normal development, some cells occupy an intermediate, or “hybrid,” state, where they express genes characteristic of more than one cell type. The eventual fates of these hybrid cells and the mechanisms behind their formation remain unknown. We are studying hybrid cells within the axial mesoderm of early zebrafish embryos as a model to understand the behaviors and fates of hybrid cells in development. Early in development, cells in the axial mesoderm share a common progenitor that expresses a single set of genes. As development continues, the anterior portion of the axial mesoderm responds to different signaling factors than the posterior, distinctly specifying cells in both regions. By 7 hours post fertilization (hpf), the anterior is specified into the prechordal plate (PCP) and the posterior is specified into the notochord. However, at this timepoint, a small population of “hybrid” cells at the boundary between the cell types expresses genes specific to both notochord and PCP identities. Here, we characterize the fate of these hybrid cells and using new notochord transgenic lines and hybridization chain reactions (HCR). Fate-mapping using these lines shows that at least some hybrid cells with former notochord expression contribute to the hatching gland, the final-destination of prechordal plate cells. We identified that these hybrid cells eventually differentiate into prechordal plate cells, although they are delayed in this differentiation compared to non-hybrid prechordal plate cells. We aim to characterize the fate and potential functionality of these cells further using single-cell genomics approaches and to investigate the gene network that regulate their development through mutations and signaling molecule manipulation. Additionally, we plan to perform a parallel set of experiments to characterize hybrid cells that end up outside of the PCP. Funding: NIH Intramural ZIAHD008997 to JAF

1074T **Generating transgenic zebrafish for optogenetic control of signaling pathways** William Anderson, Leanne Iannucci, Katherine W Rogers NICHD, National Institutes of Health

A fascinating biological phenomenon is the development of a single cell into a mature organism with a diverse range of tissues and cell types. Signaling pathway activity is crucial to this development. To better understand how signaling generates diverse cell types, we use optogenetic tools to activate the FGF, Nodal, and BMP pathways in zebrafish. Previously, we have used mRNA microinjection to introduce these tools into one-cell embryos, a technique which can be time consuming and result in mosaicism. We now seek to generate transgenic zebrafish lines that express these tools, which should greatly facilitate further experimental pathway manipulation by eliminating the need for microinjections and providing more ubiquitous expression. Some anticipated challenges we are working to overcome include fine-tuning expression levels and housing potentially light-sensitive adult zebrafish. We believe these transgenics will improve the ease and precision of optogenetic signaling manipulation and facilitate longer term experiments than those currently allowed by mRNA microinjection. Funding: NIH Intramural ZIAHD009002-01 to KWR.

1075T **Wnt-signaling dependent mechanisms of zebrafish spinal cord regeneration** Sam Alper[1], Deeptha Vasudevan[2], Maya Wheeler[1], Richard Dorsky[1] [1]Neurobiology, University of Utah, [2]University of Chicago

Spinal cord injury (SCI) leads to severed axons, neuronal death, and a resulting loss of sensorimotor function. In mammals, damaged axons are not efficiently regrown and no new neurons are born after injury. An important barrier to mammalian

spinal cord regeneration is provided by the injury-induced response of non-neural cells. Several cell types including fibroblast, immune, and meningeal cells infiltrate the mammalian injury site and secrete an array of molecules into the extracellular matrix. The resulting fibrotic scar contains inhibitory signals and creates a physical obstacle to regrowing axons. These cellular and molecular responses lead to permanent deficits in sensorimotor function. Zebrafish, however, respond to spinal cord injury with axon regrowth, neurogenesis, and functional recovery. This remarkable ability is underpinned in part by an upregulation of signaling pathways associated with development, including Wnt/β-Catenin. Prior data from the Dorsky lab and others show the Wnt/β-Catenin signaling cascade is required for both axon growth and neurogenesis. However, the cell types that respond to Wnt signaling and the Wnt target genes that promote regeneration remain poorly characterized. Our RNA-seq data show Wnt-dependent regulation of several meningeal marker genes as well as the pro-regenerative fibroblast markers *prrx1a/b*. While Prrx1 has a demonstrated role in regeneration and wound healing across several tissues, a role in nervous system repair has not yet been investigated. Further expression analysis shows that these transcripts are injury-responsive and require Wnt-signaling for their expression. Future work on this project will determine whether meningeal cells, Prrx1+ fibroblasts, and their respective candidate genes, are required for functional recovery after SCI.

1076T **Investigating the Role of Cold-Inducible RNA-Binding Proteins A and B (Cirbpa/b) in Balbiani Body Assembly and Translational Repression of Maternal RNAs** Megan N Guerin, Allison H Jamieson-Lucy, Mary C Mullins Cell and Developmental Biology, University of Pennsylvania

For most vertebrates, axial patterning and germline specification during early embryogenesis depend on establishing polarity within the oocyte. The Balbiani body, an aggregate of maternal RNAs, protein, and membrane-bound organelles, initiates polarity by disassembling at the oocyte cortex. Upon fertilization, disassembled components of the Balbiani body translocate towards the blastomeres and have two notable functions: to induce germ cell fate in the cells that inherit these components and to specify the dorsal organizer, the signaling center that orchestrates dorsal-ventral patterning during gastrulation. It is speculated that an additional role of the Balbiani body is to repress the premature translation of maternal RNAs. Although the Balbiani body is indispensable in acquiring polarity, the molecular mechanisms governing its assembly and role in the translational repression of maternal factors remain elusive. Buc is the only known protein to be required for Balbiani body assembly, which in *bucky ball* mutant females fails to form, resulting in polarity defects that cause embryonic lethality. Biochemical analysis revealed that Balbiani body assembly during oogenesis depends on the self-aggregation properties of the Buc prion-like domain. Our lab has discovered that the zebrafish Balbiani body proteome contains three proteins predicted via bioinformatics analysis to possess prion-like domains: Buc and Cold-Inducible RNA-Binding Proteins A and B (Cirbpa/b). Cirbpa/b are excellent candidates to modulate Balbiani body assembly and function as they possess both a prion-like domain and an RNA-recognition motif within their protein structure, which suggests that they have self-aggregating and RNA-binding abilities. We have generated a mutant Cirbpa CRISPR allele and obtained a mutant Cirbpb allele from the Zebrafish Mutation Project to further investigate the role of Cirbpa/b in Balbiani body assembly and translational repression of maternal RNAs during oogenesis. I will report on my analysis of maternal Cirbpa/b double mutants and observed Balbiani body defects. This research is supported by NIH NIGMS award number R35GM131908.

1077T **A zebrafish gill model of mammalian lung endothelium** Jong Park[1,2], Celia Martinez-Aceves[1], Daniel Castranova[1], Louis Chip Dye[1], Chris Dell[3], Melissa Mikolaj[3], Madeline Kenton[1], Gennady Margolin[1], Van Pham[1], Kedar Narayan[3], Brant Weinstein[1] [1]NICHD/NIH, [2]NIGMS/NIH, [3]NCI/NIH

Endothelial cells (ECs) are a critical component of the lungs and are essential for regulating gas/electrolyte exchange and immune responses. Dysregulation of ECs in the lungs is involved in major cardiopulmonary diseases. Recent studies in mice identified a novel population of lung ECs, “Aerocytes,” that likely play a key role in gas exchange and respiratory pathophysiology. However, the developmental origins and specific functions of these unusual cells are still unknown due to difficulties in observing and manipulating them in vivo in mammalian lungs. Although zebrafish gills function in an aqueous environment, they facilitate gas exchange and share functionally equivalent cell types with lungs. Unlike mammalian lungs, however, externally situated zebrafish gills are readily accessible for high-resolution optical imaging and experimental manipulation, making the fish an excellent comparative model for studying the origins and function of gas exchange endothelium in vivo. We have performed confocal imaging of EC transgenic reporter lines and transmission electron microscopy to characterize gill vascular development and morphology. We have also carried out scRNAseq on dissociated adult zebrafish gills, identifying several distinct novel populations of ECs in the gill, including ECs with a molecular profile that strongly resembles mammalian Aerocytes. In situ hybridization chain reaction (HCR) revealed that zebrafish Aerocytes localize to the highly vascularized lamella of the gill where gas exchange is taking place. We are currently generating transgenic lines marking these cells to better characterize their developmental origins and function. Together, our findings will help establish a new experimentally accessible comparative vertebrate model for studying the development and pathologies of mammalian lung gas-exchange endothelium. This research was funded by the NICHD Intramural research program (to BMW).

1078T **Investigating the role of cytoskeletal dynamics during epiboly morphogenesis in the zebrafish** Bakary Samasa[1], Joe Zinski[2], Mary C. Mullins[2] [1]Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, [2]Perelman School of Medicine at the University of Pennsylvania

Epiboly is the first coordinated morphogenetic cell movement during zebrafish development, wherein the cells of the embryo spread over and envelop the yolk. These morphogenetic movements are essential to the transition from a group of individual cells to the formation of a body plan during development. Thus, the dynamics and regulation of epiboly remains a fundamental question in biology. Epiboly is initiated by yolk cell doming which is coupled to oriented cell divisions that lead to an expansion of an epithelial enveloping cell layer (EVL). However, the mechanism/s of epiboly progression remain unclear. Here, we investigate the roles of p38 mitogen activated kinase (MAPK) and its substrate Mapkapk2 in epiboly progression. Embryos from homozygous maternal-effect *mapkapk2* null mutant females and dominant-negative p38 expressing embryos exhibit a defect in epiboly wherein the blastoderm margin constricts circumferentially beginning at the 50% epiboly stage, causing the yolk cell to burst. It has been shown that Mapkapk2 functions in the yolk cell to regulate epiboly progression, where it is hypothesized to modulate actomyosin-based contractility. Here I use live and fixed imaging of actin to investigate cytoskeletal dynamics to understand the role of actin dynamics in mediating Mapkapk2 and p38 driven epiboly progression. These initial results set the stage for further investigation of epiboly dynamics in other mutants and fluorescent reporter lines of the cytoskeleton and adhesion factors.

1079T **Investigating Fer Kinase expression during brain development in zebrafish** Madison Korkeakoski, Marissa Bell, Aaron Putzke Whitworth University

Fer kinase is a non-receptor tyrosine kinase that has been highly conserved during evolution across invertebrates and vertebrates alike. This protein is known to play a role in cell-cell adhesion, cell proliferation, and has been linked to the regulation of cancer cell progression and survival. While it is known that Fer is generally required for proper development, in vivo functions of Fer during various developmental stages are not widely described. Previously, we have shown that Fer is required for normal red blood cell development in embryonic zebrafish, in addition to demonstrating important kinase independent functions of Fer that require it for normal vasculature organization. In this study, we demonstrate Fer Kinase expression in the brain during zebrafish embryogenesis with a focus on retinal and neural tube formation, visualized through whole mount in situ hybridization and cryosectioning, as well as knock-down experiments using morpholino analysis.

1080F **Characterizing the role of a novel sperm-supplied protein, SPSP-1, during spermatogenesis and early development in Caenorhabditis elegans** Darline Murat[1], Nancy Duker[2], Ji Kent Kwah[1], Frank Doring[3], Aimee Jaramillo-Lambert[1] [1]University of Delaware, [2]Rutgers University, [3]University of Kiel

SPE-11 is a spermatogenesis specific protein that produces a paternal effect lethal phenotype when mutated (Browning et al.1996). spe-11 mutations have been linked to improper egg activation including defects in eggshell formation, oocyte polar body formation, failure in the first mitotic spindle orientation, and incomplete cytokinesis (Browning et al.1996; L'Hernault et al. 1988; Jaramillo-Lambert & Golden, 2020; McNally & McNally, 2005). In an effort to find interacting partners of SPE-11, a Yeast 2 Hybrid screen was performed and spsp-1 (Sperm Partner of SPE-11) was identified as a potential interactor. spsp-1 is a novel gene, thus the function of SPSP-1 and how it interacts with SPE-11 are still unknown. Two spsp-1 deletion mutant lines were created via CRISPR/Cas9 genome editing (spsp-1Δ1 and spsp-1Δ2). Both spsp-1Δ1 and spsp-1Δ2 have reduced brood sizes at 24℃ compared to wild type. However, wild-type sperm was able to rescue this reduction in brood size indicating a sperm defect. To determine if SPSP-1 plays a role in eggshell formation similar to SPE-11, we performed eggshell permeability assays. The results showed that spsp-1Δ1 and spsp-1Δ2 do not have significant eggshell defects. We also performed localization studies using an N-terminal GFP-tagged SPSP-1 and found that SPSP-1 is expressed in the male germline and colocalizes with SPE-11 during spermatogenesis. Future directions of this study include studying whether SPSP-1 and SPE-11 are reciprocally required for proper localization and epistasis analysis and ultimately determine how SPSP-1 and SPE-11 function in spermatogenesis and egg activation.

1081F **Parallel roles of PAR-1 and UBA-2 in the C. elegans intestine** Melissa Pickett, Zoe Upham Biological Sciences, San Jose State University

Apico-basolateral polarity establishment and maintenance are essential for the function of the epithelial cells that line the organs of animals and act as selective barriers and transporters. Disruption of apico-basolateral polarity is associated with many diseases, including microvillus inclusion disease, polycystic kidney disease, and the progression and metastasis of cancers. The mechanisms underlying apico-basolateral polarity are likely to be highly redundant due to the importance of apico-basolateral polarity for organ function. Indeed, the loss of many polarity proteins individually from epithelial tissues often has only minor effects on the structure and function of the epithelium. Our goal is to elucidate the mechanisms by

which apico-basolateral polarity is established and maintained, using the *C. elegans* intestine as a simple *in vivo* model. We are particularly interested in testing the hypothesis that the conserved polarity kinase, PAR-1/MARK, acts in parallel with UBA-2, a protein involved in SUMOylating pathways, as little work has focused on the role of SUMOylating pathways in apico-basolateral polarity establishment and maintenance. In previous studies, PAR-1 and UBA-2 were redundantly required for the establishment of anterior-posterior polarity in the one-cell *C. elegans* embryo. In a preliminary RNAi screen, we similarly found that intestine specific depletion of PAR-1 (PAR-1$^{gut(-)}$) along with RNAi depletion of *uba-2* arrested worm development while neither PAR-1$^{gut(-)}$ nor *uba-2 RNAi* alone affected worm growth. Therefore, we plan to determine whether UBA-2 is involved in apico-basolateral polarity establishment and maintenance and whether it works redundantly with PAR-1.. We are currently working to endogenously tag UBA-2 with a zinc finger degradation domain and GFP to describe the localization of UBA-2 for the first time. This strategy will also allow us to degrade UBA-2 in a tissue specific manner without affecting its potential roles in other tissues. We will further be able to concurrently deplete both PAR-1 and UBA-2 from the intestine to determine if the two proteins act redundantly in apico-basolateral polarity establishment and/or maintenance Here, we will report on the localization of endogenous UBA-2 in the *C. elegans* embryo and larval stages. We will further report on the growth and localization of polarity proteins in worms depleted of intestinal PAR-1, UBA-2, or simultaneously depleted of both PAR-1 and UBA-2 compared with controls.

1082F **A bacterial genetic screen to identify how diet regulates *C. elegans* germline stem cells** Katherine X Norton[1], Elisa Taine[1,2], Julia Burnett[1], E. Jane Albert Hubbard[3] [1]NYU Grossman School of Medicine, [2]Université Paris Cité, [3]Cell Biology, NYU Grossman School of Medicine

Stem cells are capable of self-renewal and of producing cells that differentiate, and they can respond to changes in an organism's environment such as the quantity and quality of its diet. In many stem cell systems, the stem vs. differentiated cell fate decision is controlled via interaction with a stem cell niche. In the *C. elegans* germ line, ligands for the Notch receptor are expressed in the niche, the distal tip cell (DTC), which activate a Notch receptor (GLP-1) in neighboring germ cells to maintain stem cell fate.

Previously, our lab found that DAF-7 TGFß, the neuronal expression of which is modulated by diet, acts through its receptor DAF-1 on the DTC such that diet alters the expression of *lag-2,* a ligand for Notch, thereby modulating the number of germline stem cells that accrue during larval stages (Dalfó et al., 2012 *Current Biology*; Pekar et al., 2017 *Development*). However, we do not know the specific components of the *C. elegans* diet that act on this TGFß "neuron-to-niche" axis to regulate germline stem cell fate.

To identify key dietary components, we screened through the Keio *E. coli* mutant library for mutants that when fed to worms, affected a read-out of the neuron-to-niche axis. In a prior study (Venzon et al., 2022 *Cell Host and Microbe*), the readout was a fertility assay. Although the screen generated interesting hits, none were related to expression of *lag-2* in the DTC. Therefore, we developed a new screening strategy. In high through-put 96-well liquid format, we tracked a *lag-2* promoter-driven DTC marker that is visible in wild-type worms but is not visible when the DAF-7 TGFß axis is perturbed. Counts of both worms and detectable DTCs were automated, and we developed a pipeline for scoring that considered the density of the bacteria and the number of scorable worms. Secondary screening tested candidate bacterial mutants for robust reproducibility. Primary screening identified 317 candidates from the Keio library (3,985 mutants). From these, one candidate emerged from the secondary screen that reduced detection of the *lag-2*-driven reporter in the DTC. We validated that this Keio library mutant deletes the gene *sodB* (*ΔsodB*). *SodB* encodes a superoxide dismutase enzyme and is one of three superoxide dismutases in *E. coli*. We are testing several hypotheses for how *ΔsodB* might impair expression of the *lag-2* reporter, and whether the effect is dependent on TGFß signaling.

1083F **The Secreted Protein SPE-36 is Dependent on Multiple Other Proteins for Proper Localization in *C. elegans* Sperm** A›Maya Looper, Amber Krauchunas Biological Science, University of Delaware

Fertilization is the process by which sperm and egg fuse to give rise to a zygote. Defects in fertilization can result in sterility. We use the model organism *C. elegans* to study a sperm-specific fertilization defective mutant known as *spe-36. spe-36* belongs to a group of 10 sperm-specific genes required for fertilization. The loss of any single one of these genes renders the worm sterile, but otherwise a healthy adult. Sperm from the mutant are morphologically identical to that of wild-type sperm, however, they fail to fertilize an egg. Together with proteins on the egg›s surface, these sperm proteins help form the fertilization synapse.

In WT, SPE-36 localizes to the membranous organelles (MOs) of spermatids. In spermatozoa, SPE-36 localizes to the pseudopod of the active sperm and the cell body. Because SPE-36 is a secreted protein, we hypothesize that it requires a binding partner to keep it associated with the pseudopod of spermatozoa. We set out to test if SPE-36 localization is dependent on other

sperm-specific fertilization proteins. In order to test this, we are crossing SPE-36::GFP into mutant backgrounds of each of the 9 other proteins. Thus far we have crossed SPE-36::GFP into five different mutant backgrounds: *spe-9, spe-38, spe-13, spe-42* & *spe-49*. Live imaging data shows that SPE-36 localization is dependent on *spe-9, spe-38, spe-13, spe-42* & *spe-49*. In each mutant background, SPE-36 fails to localize to the pseudopod.

These data adds more knowledge to the field and further supports the model that multi-protein complexes help mediate gamete interactions and fusion. In the future, we aim to test if SPE-36 localization is similarly dependent on the other four known sperm-specific fertilization proteins.

1084F **The role of spectrin-mediated mechanotransduction in the morphogenesis of a large extracellular structure in *C. elegans*** Prioty F Sarwar[1], Trevor J Barker[2], Meera V Sundaram[1] [1]Genetics, University of Pennsylvania, [2]University of Pennsylvania

The apical extracellular matrix (aECM) lines, shapes and protects exposed surfaces in animals and often organizes to form elaborate, acellular structures like the scales of a butterfly wing or *Drosophila* denticles. How animals pattern these large structures during development is poorly understood, in part due to the immense diversity of these anatomical features found in the animal kingdom. The *C. elegans* alae, ridges that run along the sides of the worm in three long 'racing' stripes, are an example of one such extracellular structure. Here, we study how a set of cytoskeletal proteins organize at the lateral epidermis at the last larval stage of *C. elegans* development for the *de novo* formation of the adult alae.

Previous research in the lab found that four longitudinal actin filament bundles (AFBs) form at the lateral epidermis, similar to the AFB organization reported in patterning butterfly wing scales or *Drosophila* bristle ridges. We also found that a transient aECM known as the pre-cuticle is required for alae formation. Additionally, ultrastructural data revealed a potential role of pre-cuticle delamination in alae morphogenesis, where four small horizontal areas of delamination demarcate the valleys observed between the developing alae. One hypothesis generated by this observation is that the longitudinal AFBs exert mechanical forces on the overlying matrix to trigger pre-cuticle delamination.

We tested several cytoskeletal proteins that have actin binding and plasma membrane binding domains, making them ideal candidates to aid in the assembly of AFBs at the lateral epidermis and/or facilitate AFB-mediated mechanical forces on the matrix. Super-resolution imaging shows that the spectraplakin VAB-10 and intermediate filament bundles are arranged in a single line at the middle of the lateral epidermis offset from AFBs, while the βH-spectrin SMA-1 is found superimposed on the AFBs. Additionally, *sma-1* null mutant worms show disorganized pre-cuticle and alae structure and reduced mechanosensitive protein localization at the lateral epidermis. Overall, our data support the hypothesis that mechanical forces relayed by cytoskeletal structures play a role in the formation of the *C. elegans* adult alae.

1085F **The role of different PAR-3 domains in establishing and remodeling apico-basolateral polarity across the intestine** Mariam Mortada, Alyza J Escuadro, Adam Brewer San Jose State University

The polarized localization of proteins to specific cellular domains is essential for the function of many cell types, including the epithelial cells that line the organs of all animals. This polarity is critical in creating barriers against pathogens, directing transport of molecules, and providing mechanical resilience to organs, and loss of polarity can lead to diseases including cancerous tumor formation and metastasis. PAR-3 is a conserved scaffolding protein that is required for polarity establishment across epithelial tissues of many species. Previous work using continuous intestine specific depletion of PAR-3 in C. elegans demonstrated that PAR-3 was necessary for the correct timing of polarity establishment and for the formation of a continuous hollow lumen. PAR-3 was found to be dispensable during polarity maintenance in larval worm stages. Surprisingly, when we expressed PAR-3 in the first larval stage (L1) after depleting PAR-3 during polarity establishment in embryonic stages, we found that the cystic intestines were remodeled leading to continuous hollow lumens and correctly oriented apical-basolateral polarity axes in ~30% of the worms. We are currently interested in determining the efficacy of this recovery and in determining which domains of PAR-3 are necessary for the establishment and remodeling of apico-basolateral polarity across epithelial tissues, using the C.elegans intestine as a model. We are testing the hypothesis that the same domains of PAR-3 are necessary in coordinating apico-basolateral polarity establishment and in the observed remodeling. Therefore, we deleted specific domains (PDZ 1-3, PDZ-1, PDZ-2, PDZ-3, the oligomerization domain, or CR3) from PAR-3 and are expressing them either during polarity establishment or in the larval remodeling phases in worms in which endogenous PAR-3 is continuously depleted to determine if the mutated versions are capable of rescuing polarity establishment and intestinal remodeling.

1086F **Establishing an auxin-inducible degron system to dissect spatiotemporal function of GLD-2** Karl-Frederic Vieux[1], Graciela Galvez[2,3], Katherine McJunkin[4] [1]Laboratory of Cellular and Developmental Biology, National Institute of Health, [2]National Institutes of Health, [3]Department of Biology, Colgate University, [4]Laboratory of Cellular and Developmental Biology, National Institutes of Health

RNA translation and stability are critical to reproduction and development. Tailing is the addition of nucleotides to the 3' end of RNA molecules in an untemplated manner. It is mediated by terminal nucleotidyl transferases (TENTs), and it modulates RNA translation and stability. Historically, long poly(A) tails have been associated with stable and highly translated transcripts. However, new high-throughput sequencing methods show the incorporation of non-A-residues in tails and suggest that both nucleotide composition and length determine tail function. RNA tails in the oocyte and early embryo are particularly dynamic, yet our understanding of their function and requirement in oogenesis is limited. GLD-2 is a noncanonical TENT that specialize in cytoplasmic poly(A) tailing. Global loss of *gld-2* prevents the initiation of meiosis and a complete loss of oocytes in the *C. elegans* germline. Here, the auxin-inducible degron (AID) system was used to conditionally deplete *gld-2* expression in the germline of hermaphrodite adults. Unlike a global knockout of *gld-2*, germline-specific depletion of GLD-2 in adults did not prevent the initiation and progression of meiosis: diakinetic oocytes were observed at the distal end of gonads for several days after auxin treatment. This difference may be explained by the broad timing and the ubiquitous nature of a *gld-2* depletion in a null mutant. Yet, triggering the depletion of GLD-2 at larval stages did not recapitulate null phenotypes, nor did a ubiquitous depletion of GLD-2. Within 4hr of auxin induced GLD-2 depletion, these conditional mutants exclusively lay dead embryos that arrest at the early stages of embryogenesis, phenocopying long term *gld-2* RNAi experiments. Fertilization by wild-type sperm does not rescue this embryonic defect. Future experiments will determine the exact developmental and tissue-specific GLD-2 requirements for germline establishment and the initiation of meiosis using the AID system. The acute spatiotemporal control of GLD-2 expression with this system can then be leveraged to broadly identify GLD-2 functional targets in oocytes and early embryos. A novel direct cDNA sequencing method (Nano3P-seq) will be used to accurately resolve mRNA tail length and composition in the absence of GLD-2. These results will shed light on the cellular and physiological implications of RNA tailing and identify molecular pathways that determine terminal oocyte differentiation and embryonic viability.

1087F **Identifying genes involved in the *kin-20* developmental pathway in *C. elegans* through a forward genetic screen** Jadan Hand[1], Vivian Viacobo[2], Michelle Coluzzi[1], Priscilla Van Wynsberghe[1] [1]Biology, Colgate University, [2]Colgate University

C. elegans exhibit rhythmic processes and express an ancestral form of the circadian clock which impacts development and developmental timing. Central to this clock is the transcription factor, Period, whose homolog in *C. elegans* is LIN-42. Like other Period proteins, LIN-42 levels oscillate throughout development. This oscillation pattern is thought to be due in part to the putative kinase KIN-20, whose homolog is Doubletime in Drosophila and CK1ε in humans. Prior work in our lab has shown that *kin-20(ok505) C. elegans* exhibit decreased fertility, a slow growth rate, and an uncoordinated phenotype. Thus, KIN-20 likely impacts other targets beyond LIN-42 to play a significant role in organismal development. To better understand this role, we have begun a forward genetic screen to identify suppressors of these *kin-20(ok505)* phenotypes. To do this, *kin-20(ok505)* worms were exposed to ethyl methanesulfonate (EMS), and F2s were screened for suppressors of the *kin-20(ok505)* delayed growth and movement phenotypes. Populations of suppressors were then more systematically compared to WT N2 worms to identify top candidates for further analysis. Current work aims to further characterize these candidates before identification of mutations by whole-genome sequencing. Altogether this work will increase our understanding of the various functions that this important, conserved gene plays in development.

1088F **Expanded roles for LOTR-1 in the *C. elegans* germline** Noah A Lind[1], Elisabeth Marnik[2], Catherine Sharp[1], Dustin Updike[1] [1]Mount Desert Island Biological Laboratory, [2]College of Science and Humanities, Husson University

LOTUS and Tudor domain-containing proteins play a crucial role in forming biomolecular condensates that support the inheritance of small RNA and germ cell functionality.[1] One such protein, the *C. elegans* TDRD5 homolog LOTR-1, interacts with the helicase ZNFX-1 in cytoplasmic Z-granules to ensure the even distribution of small RNAs across WAGO and Mutator targets.[2] Interestingly, LOTR-1 recruits two essential subunits of the *nuclear* CstF 3'UTR cleavage and polyadenylation complex, CPF-1 and SUF-1, to the *cytoplasmic* LOTR-1/ZNFX-1/WAGO complex.

In Xenopus oocytes, SUF-1/CstF-77 is primarily nuclear but is also found in foci outside the nucleus that contain cytoplasmic polyadenylation proteins (e.g., GLD-2) and the cap-binding initiation factor eIF4E; however, the function of cytoplasmic CstF-77 is unclear and may potentially reduce translation efficiency.[3] Here, we look closely at the nuclear and the cytoplasmic retention of SUF-1-tagged reporters in wild-type and domain-specific *lotr-1* mutants. Localization studies are complemented with total RNAseq and polysome-RNAseq in wild-type and domain-specific *lotr-1* mutants to compare differential expression, differential translation, and identify potential differences in 3'UTR modifications. These investigations aim to clarify LOTR-1's role in the germline and elucidate the function of nuclear CstF factors retained in cytoplasmic condensates.

1. Kubíková, J., Reinig, R., Salgania, H. K. & Jeske, M. LOTUS-domain proteins - developmental effectors from a molecular perspective. *Biol Chem* **402**, 7–23 (2020).

2. Marnik, E. A. *et al.* The Caenorhabditis elegans TDRD5/7-like protein, LOTR-1, interacts with the helicase ZNFX-1 to balance epigenetic signals in the germline. *PLoS Genet* **18**, e1010245 (2022).

3. Rouget, C., Papin, C. & Mandart, E. Cytoplasmic CstF-77 protein belongs to a masking complex with cytoplasmic polyadenylation element-binding protein in Xenopus oocytes. *J Biol Chem* **281**, 28687–98 (2006).

1089F **Dissecting the gene regulatory networks controlling temporal dynamics of post-embryonic C. elegans somatic gonad development** Brian Kinney[1], Fred Koitz[1], Jason Menjivar[2], Ryan Merritt[1], Christopher Hammell[3], Kacy L Gordon[1] [1]Biology, University of North Carolina, [2]University of North Carolina, [3]Cold Spring Harbor Laboratory

C. elegans larval development consists of four molts in which major genetic and cellular events must be precisely coordinated to shed and regrow the cuticle covering the animal's body. The major genetic regulators of larval developmental timing were identified with the discovery of heterochronic mutants, animals that either continuously repeat or skip developmental events during the larval stages. The genes that make up the core of the developmental timer generate oscillatory expression of *lin-4* miRNA every molting cycle; my recent work revealed that this oscillation is controlled by the *C. elegans* orthologs of highly conserved circadian regulators *nhr-23* (ROR), *nhr-85* (Rev-erb), and *lin-42* (Period). The larval skin operates under this oscillating developmental timer as it undergoes repeated cycles of molting and regrowth, characterized by pulsatile expression of *lin-42*/Period. However, the somatic gonad still develops in a *lin-4* mutant along its linear timeline of migration led by the distal tip cell (DTC), which also acts continuously as the germline stem cell niche. I have discovered that the oscillation of *lin-42* can nevertheless be observed in the DTC with the same period as the pulses in larval skin, suggesting common regulation across tissues. Yet, we found that the developmental regulators of *lin-42* in the larval skin (*nhr-85* and *nhr-23*) are dispensable for proper DTC migration. We set out to discover the regulators and role of the developmental genetic timing system in the DTC, where its known regulators (*nhr-23* and *nhr-85*) and primary target (*lin-4*) are dispensable for proper migration. DTC migration is known to be regulated in part by heterochronic factors such as *lin-42*, *blmp-1*, and *lin-29*, but their transcriptional relationships are not known. Additionally, while *blmp-1* was thought to act only as a repressor in the DTC, recent studies in other cells have shown that it can also act as a pioneer factor. We have found that *blmp-1* acts as a pioneer factor in the DTC and is necessary for localization of integrins necessary for DTC migration. Using yeast 1-hybrid screening we have set out to find the candidates that transcriptionally link *blmp-1* to the effectors of DTC migration. Using genetic techniques and live-imaging confocal microscopy we will uncover the transcriptional gene regulatory networks that regulate DTC migration timing to understand how timing of developmental processes are synchronized across different tissues of the body.

1090F **MLT-11 is necessary for C. elegans embryogenesis and apical extracellular matrix structure** James M Ragle, Ariela Turzo, John C Clancy, Keya Jonnalagadda, Anton Jackson, Max T Levenson, An A Vo, Vivian Pham, Jordan D Ward Molecular, Cell, and Developmental Biology, UC Santa Cruz

Apical extracellular matrices (aECMs) are associated with all epithelia and form a protective layer against biotic and abiotic threats in the environment. Despite their importance, we lack a deep understanding of their structure and dynamics in development and disease. C. elegans molting offers a powerful entry point to understanding developmentally programmed aECM remodeling. A transient matrix is formed in embryos and at the end of each larval stage, presumably to pattern the new cuticle. A number of proteases and protease inhibitor genes are required for this process but their exact functions remain poorly understood. Focusing on targets of NHR-23, a key transcription factor which drives molting, we identified the Kunitz family protease inhibitor mlt-11 as an NHR-23 target. MLT-11 is an unusual predicted protease inhibitor with 10 Kunitz domains. We identified NHR-23-binding sites that are necessary and sufficient for epithelial expression. We generated internal and C-terminal MLT-11::mNeonGreen::3xFLAG (MLT-11::mNG) knock-ins through CRISPR which displayed distinct localization patterns. The C-terminal fusion displayed weak localization in the cuticle and strong lysosomal localization. In contrast, the internal knock-ins displayed more robust cuticular localization with a striking pattern lining the cells of the vulval lumen. Western blot analysis suggested that MLT-11 is processed into two fragments and structure function analysis implicates defects in the N-terminal fragment in a right roller phenotype while the defects in the C-terminal fragment cause embryonic lethality. Deletion of parts of both the N-terminal and C-terminal fragments produced a synthetic phenotype with severe developmental delay, cuticle blistering, and small body size. Tissue-specific RNAi suggests that mlt-11 activity is primarily necessary in seam cells and we observe seam cell fusion defects when we deplete mlt-11. Together, these data show that MLT-11 plays multifunctional roles to coordinate aECM structure and function during development.

1091F ***Sperm-specific sterility conferred by XND-1 paralogs in* Caenorhabditis elegans** Sharon Li[1], Cristina Quesada Candela[2], Judith L Yanowitz[3] [1]Magee-Womens Research Institute, University of Pittsburgh, [2]Chromosome Biology, University of Vienna-Vienna BioCenter, [3]OBGYN, Magee-Womens Research Institute, U. Pittsburgh Sch. of Medicine

The chromatin factor X-nondisjunction factor (XND-1) plays important roles in double strand breaks (DSBs) during meiotic

crossover formation and in regulating germline differentiation. XND-1 has two distant paralogs in *C. elegans*, Y54G2A.26 and Y54G2A.73, that we have nicknamed XLP-1 and XLP-2 (XND-1-like proteins-1, -2) for simplicity. There was no known mutation for *xlp-1*, so we created a complete knock-out of the coding region using CRISPR. We obtained a deletion allele of *xlp-2* from the CGC. Mutations in both *xlp-1* and *xlp-2* were found to be sperm sterile mutants (SPE): hermaphrodites are self-sterile but can be rescued by outcrossing; males are sterile. Through further testing, we found that *xlp-1* sperm fail to undergo spermiogenesis and exhibit defects during the meiotic cell divisions. Our progress in characterizing the defects in both sets of mutants and in determining protein localization will be presented.

1092F **CED-5/CED-12 (DOCK/ELMO) can promote and inhibit F-actin formation via distinct motifs that target different GTPases** Yeshaswi Pulijala, Thejasvi Venkatachalam, Kavya Kadabageri, Maria Dawood, Rose Swan, Martha Soto Pathology and Laboratory Medicine, Rutgers (RWJMS)

Coordinated activation and inhibition of F-actin supports the movements of morphogenesis. Understanding the proteins that regulate F-actin is important, since these proteins are mis-regulated in diseases like cancer. Our studies of *C. elegans* embryonic epidermal morphogenesis identified the GTPase CED-10/Rac1 as an essential activator of F-actin. However, we need to identify the GEF, or Guanine-nucleotide Exchange Factor, that activates CED-10/Rac1 during embryonic cell migrations. The two-component GEF, CED-5/CED-12, is known to activate CED-10/Rac1 to promote cell movements that result in the engulfment of dying cells during embryogenesis, and a later cell migration of the larval Distal Tip Cell. It is believed that CED-5/CED-12 powers cellular movements of corpse engulfment and DTC migration by promoting F-actin formation. Therefore, we tested if CED-5/CED-12 was involved in embryonic migrations, and got a contradictory result. CED-5/CED-12 definitely support embryonic migrations, since their loss led to embryos that died due to failed epidermal cell migrations. However, CED-5/CED-12 inhibited F-actin in the migrating epidermis, the opposite of what was expected for a CED-10 GEF. To address how CED-12/CED-5 could have two opposing effects on F-actin, during corpse engulfment and cell migration, we investigated if CED-12 harbors GAP (GTPase Activating Protein) functions. A candidate GAP region in CED-12 faces away from the CED-5 GEF catalytic region. Mutating a candidate catalytic Arginine in the CED-12 GAP region (R537A) altered the epidermal cell migration function, and not the corpse engulfment function. A candidate GEF region on CED-5 faces towards Rac1/CED-10. Mutating Serine-Arginine in CED-5/DOCK predicted to bind and stabilize Rac1 for catalysis, resulted in loss of both ventral enclosure and corpse engulfment. Genetic and expression studies showed the GEF and GAP functions act on different GTPases. Thus, we propose CED-5/CED-12 support the cycling of multiple GTPases, by using distinct domains, to both promote and inhibit F-actin nucleation. We are investigating how CED-5/CED-12 are recruited to distinct membranes. In addition, we are characterizing additional Guanine-nucleotide Exchange Factors that regulate morphogenesis through the Rac1 GTPase.

1093F **From Migration to Mature Elaboration: Exploring *C. elegans* Stem Cell Niche Transformation via Morphology and Transcriptomics** Xin Li[1], Kin Gomez[2], Adriana San Miguel[2], Kacy Gordon[1] [1]Biology, University of North Carolina, Chapel Hill, [2]North Carolina State University

The *Caenorhabditis elegans* hermaphrodite distal tip cell (DTC) acts as the germline stem cell niche and has served as a model for cell migration and stem cell niche biology. DTCs migrate along a stereotyped path, lead gonad elongation, and determine gonadal morphology during post-embryonic development. At the larval-to-adult transition, the DTC undergoes dramatic morphological changes and grows long processes that wrap adjacent germline stem cells in the distal gonad. Many genes and signaling pathways have been identified that regulate migration and niche behavior. However, how DTC transitions from its gumdrop-shaped migratory state to the elaborated mature niche is still not well understood. We are undertaking an in-depth investigation of this transformation using several approaches. At the morphological level, we are using microfluidics to record the transformation of DTCs from compact cells to large, branched cells. Microfluidics approaches mitigate the risk of germ cell quiescence due to starvation during long-term time lapsing. At the transcriptome level, we are sequencing DTC nuclei across this developmental transition to identify gene expression differences that correlate with DTC elaboration, which will furnish candidates for subsequent studies of niche migration and structure. Furthermore, we are also tagging endogenous loci and analyzing expression patterns of genes that are selectively expressed in the DTC identified in previous transcriptomic studies. By elucidating how a stem cell niche transitions from migratory to stationary and compact to elaborated, our results will provide a more comprehensive vision of genes that regulate cell migration and cell-cell interactions. Because many pathways regulating DTC niche behavior have turned out to be highly conserved in other stem cell niches, our findings will give new insights into the molecular control of niche-stem cell associations that are likely to be acting in other stem cell systems.

1094F **Identification of the spatial requirement for the DAF-2 insulin receptor in food type-dependent oogenesis onset and fertilization in *C. elegans*** Asra Akhlaq, Joy Alcedo Department of Biological Sciences, Wayne State University

The nematode worm *Caenorhabditis elegans* is highly genetically tractable and has hundreds of oocytes that can be easily

imaged *in vivo*. This makes the worm an excellent model in which to dissect the mechanisms that regulate oocyte biology in response to different diets. Recently, our lab showed that the onset of oogenesis and oocyte fertilization rates in *C. elegans* are modulated by the type of bacteria it eats (1). Our lab also found that this bacterial-dependent regulation of oogenesis and oocyte fertilization require the insulin receptor DAF-2 (1). However, the insulin-like peptide (ILP) ligand that regulates onset of oogenesis differs from the ILP(s) that regulate fertilization (1). Because the 40 ILPs in *C. elegans* are expressed in different subsets of cells (2), this raises the hypothesis that the insulin receptor, DAF-2, acts from different cells to regulate diet-dependent oogenesis versus oocyte fertilization rates. To test this hypothesis, we employ the auxin-inducible degron system (AID) to determine in which cells DAF-2 is required to modulate oogenesis or fertilization in response to the worm's bacterial diet. Identification of the cells or tissues in which DAF-2 acts to regulate these two aspects of oocyte biology will potentially delineate two different circuits through which diet regulates oogenesis onset versus fertilization.

Refs:

(1) Mishra et al. (2023). Specific sensory neurons and insulin-like peptides modulate food type-dependent oogenesis and fertilization in *Caenorhabditis elegans*. bioRxiv. doi: https://doi.org/10.1101/2022.08.31.506073 (2) Allen et al. 2015. Sensory systems: their impact on *C. elegans* survival, *Neuroscience* 296: 15-25.

1095F ***C. elegans* ALFA-1^{C9orf72}/SMCR-8 functions via ARF-6 to Negatively Regulate LET-23 EGFR via its C-terminal PDZ interaction motif.** Ahmed Sabbir[1], Aida Sobhani[1,1], Stephanie Deng[2], Kimberley Gauthier[2], Christian Rocheleau[1] [1]Anatomy and Cell Biology, McGill University, [2]McGill University

C9orf72 is the most common mutated gene associated with Amyloid Lateral Sclerosis (ALS) and Frontal Temporal Dementia (FTD). C9orf72 forms an obligate complex with SMCR8 to regulate membrane trafficking. Biochemical and structural data indicate that C9orf72/SMCR8 is a GTPase Activating Protein (GAP) for Arf and Rab GTPases *in vitro*, but the *in vivo* targets have not been identified. We have recently found that both homologues of human C9orf72 and SMCR8 in *C. elegans*, ALFA-1 and SMCR-8, negatively regulate LET-23 Epidermal Growth Factor Receptor (EGFR) signaling in an ARF-6 GTPase dependent manner. LET-23 EGFR signals via a Ras GTPase and MAPK cascade to specify vulval cell fates. LET-23 most closely resembles human EGFR family member, ErbB4/HER4, in that they share a C-terminal PDZ interaction motif (PIM). The LET-23 PIM binds the PDZ domain of LIN- 7 (mLin7) which in turn interacts with LIN-2 (CASK) which binds LIN-10 (APBA1) protein to target LET-23 to the basolateral membrane of the *C. elegans* vulva precursor cells. Mutation of the LET-23 PIM or any component of the LIN-7/2/10 complex leads to apical only localization of LET-23 and a strong vulvaless phenotype. We found that *alfa-1* and *smcr-8* deletion mutants strongly suppress the *lin-2(-)* vulvaless phenotype. Surprisingly, we found that *smcr-8* does not suppress the vulvaless phenotype of *let-23(sy1)* which specifically deletes the PIM of LET-23 or a *lin-7* mutant. To our knowledge, this is the first negative regulator of LET-23 EGFR signaling that suppresses *lin-2(-)*, but not *lin-7(-)* or *let-23(sy1).* Our data suggests that ALFA-1/SMCR-8 functions as an ARF-6 GAP to negatively regulate LET-23 EGFR signaling in a manner that is uniquely dependent on LIN-7 and the PIM of LET-23 EGFR. Considering this specific interaction, it is interesting to note that like C9orf72, ErbB4 is also mutated in familial cases of ALS and FTD.

1096F **Exploration of the roles for essential genes in C.elegans male tail tip morphogenesis using the Auxin inducible degron system** Zarifa Z Akbary, Karin Kiontke, David H.A. Fitch New York University

Morphogenesis is the universal process in the normal growth and development of animals as it involves changes in cell shape and position that must be coordinated at precise times and in specific tissues. Disruption in this process can have consequences ranging from embryonic lethality to disease. The C. elegans male tail tip provides an opportunity to address questions of morphogenesis. In males, the tail tip undergoes morphogenesis post-embryonically through the process of tail tip morphogenesis (TTM). In the larvae of both sexes, these cells form a pointed tail, but for males only, during the L4 stage, they fuse, change shape, and retract– resulting in rounded tail by adulthood. Prior findings have shown that the transcription factor, DMD-3 is required and sufficient for TTM to occur. NHR-25, a nuclear hormone receptor, is essential for the initiation of dmd-3 expression. We are addressing when exactly NHR-25 is required for TTM by using AID (auxin inducible degron system). We use a somatic promoter to drive expression of the TIR1 F-box protein in this system, allowing us to degrade NHR-25 during the L3 stage in males and measure the consequences on TTM. Preliminary data showed that there were strong male defects in the tail tip: phasmids were bludging, rays were variably fused or missing, all adult males lacked a hook, had misshapen spicules. We also found hermaphrodites were Pvul and Egl, also indicating that the degron system was effective. Similarly, we are using this system to explore the regulatory roles of other essential proteins involved in TTM, including par-3 and fos-1.

1097F **The evolution of sexually dimorphic morphogenesis.** Raya Jallad[1], Alyssa Woronik[2], Yash Patel[3], Karin Kiontke[3], David H.A. Fitch[3] [1]Biology, New York University, [2]Biology, Sacred Heart University, [3]NYU

Differences between sexes (i.e sexual dimorphism) are common in nature. Although the processes that regulate these

differences vary between taxa, a group of genes known as DM domain transcription factors (DMRTs) play a conserved role in the development of male-specific traits. However, how the development of sexual dimorphism is regulated is insufficiently known. As a model system to better understand the gene regulatory network (GRN) responsible for sexually dimorphic morphogenesis and its evolution, we study the 4 tail tip cells of *Caenorhabditis elegans.* During the last larval stage, in males only, these cells undergo a process known as Tail Tip Morphogenesis **(TTM)**, resulting in short round tail tips in males (hermaphrodites retain the pointed tips of the larvae). The DMRT transcription factor DMD-3 is required and sufficient for TTM in *C. elegans* and is hypothesized to be at the center of a bow-tie GRN (Mason et al. 2008, Nelson et al. 2011). TTM evolved multiple times independently in related nematode species, but how TTM is regulated in these species is unknown. One hypothesis for convergent evolution («hotspot» hypothesis) predicts that the architecture of GRNs biases evolution, such that the same genes/modules are co-opted when similar traits evolve repeatedly. To test this hypothesis for the convergent evolution of TTM, we are comparing TTM transcriptome profiles among several rhabditid species. As a first step in this comparison, we are characterizing transcriptome dynamics in tails of *C. elegans* males, hermaphrodites, and mutants of DMD genes using tail-tip-specific RNA-seq at several time points during the last larval stage, when TTM occurs in males. Because GRNs control cell behaviors at the transcriptional level, the key points involved in GRN evolution are proposed to be transcription factors like DMD-3, their regulation, and their interaction with target genes. Because such transcription factors generally control groups of genes, We are sorting genes into different categories of gene expression dynamic (e.g. static, upregulated, downregulated, etc.) to identify co-regulons. By comparing co-regulon profiles between species where TTM is conserved, independently gained, lost, or is ancestrally absent, we intend to identify which genes/modules are conserved and test the «hotspot» hypothesis for sexually dimorphic morphogenesis.

1098F **TORC2 in *C. elegans* Germline Development** Anke Kloock, E. Jane Albert Hubbard Department of Cell Biology, NYU Grossman School of Medicine

Certain populations of proliferating cells – from stem cells to tumors – are nutrient-responsive. Signaling pathways relay information about the surrounding environmental or organismal nutrient conditions to these pools of cells. One such pathway is the Target of Rapamycin (TOR) pathway. The TOR kinase exists in two complexes, TOR complex 1 (TORC1) and TOR complex 2 (TORC2). TORC1 has been researched extensively and its regulation, particularly by amino acids, is well characterized. In *Caenorhabditis elegans*, previous work from our lab showed that low nutrient conditions impede the establishment of the germline stem cell pool in larval stages. In one case, we determined that germline-autonomous TORC1 is required to establish the stem/progenitor pool and that nutrient conditions are relayed by germline autonomous activity of S6 kinase, a target of TORC1. By contrast, while TORC2 has been characterized for its roles in dauer entry, fat storage and body size, mitochondrial homeostasis and brood size, its role in germline stem cell development has not been previously investigated.

To begin to investigate whether and how TORC2 affects the germline stem/progenitor cell pool, and if it relays a response to nutrients, we are characterizing the role of the key TORC2 component, *rict-1*/Rictor. We generated four CRISPR/Cas9 *rict-1* null alleles and found that worms carrying these mutations fail to properly expand the germline stem/progenitor pool during development. We also noticed that the extent to which the germline stem/progenitor pool expands in *rict-1* mutants is somewhat dependent on their particular bacterial diet. Further, unlike the germline-autonomous roles of TORC1 components, the stem/progenitor pool defect of *rict-1* mutants can be rescued non-autonomously by expression of *rict-1* in the intestine alone. I am currently testing several hypotheses for how intestinal TORC2 activity regulates the size of the germline stem/progenitor cell pool, and its possible connections to nutrient availability. Due to the high conservation of TORC2 these results can have implications for how Rictor/TORC2 are non-autonomously regulating other pools of proliferating cells.

1099F **Characterization of the *C. elegans* intestinal transcriptome with spatiotemporal resolution** Justin Ellis, Robert Williams, Erin Nishimura, Jessica Hill BMB, Colorado State University

At a mere 20 cells, the *Caenorhabditis elegans* intestine functions to regulates digestion, energy homeostasis, and host defense, all while dynamically responding to its environment. It also serves to direct yolk production for the next generation and signals genetic aging. However, how these functions are regulated throughout the intestine remains unclear. The current model assumes monolithic function, uniformly performing organ-specific processes throughout the intestine and overlooks its multifaceted roles. Although sparse, research supports the spatial patterning of select transcripts among intestinal cells, lending itself to the cellular and morphological differences observed throughout the *C. elegans* intestine. Given this, we think a more accurate hypothesis is that the intestine carries out its intestinal functions heterogeneously throughout, resulting in sub-functionalized intestinal cells. Here, we seek to spatiotemporally resolve the intestinal cell transcriptome and determine if intestinal cells indeed take on specific sub-functions. To assess intestinal cell gene expression profiles, we used Fluorescence Activated Cell sorting (FACs) combined with single cell RNA sequencing (scRNA-seq). This allowed us to generate a complete transcriptional profile of the *C. elegans* intestine in a mixed stage embryo population. After identifying unique intestinal cell clusters, we then have to link them with their specific cell identity post hoc. To do this, we use small molecule inexpensive

Fluorescent In Situ Hybridization (smiFISH). This allows us to visualize individual mRNA molecules and assess their spatial expression throughout the intestine. By clustering on cellular gene expression profiles, we found that several unique intestinal cell states could be identified through scRNA-seq, supporting a heterogenous model of intestinal gene expression. From these intestinal clusters, we then selected marker genes to probe using smiFISH. One notable marker gene probed is *clec-56*, a C-type lectin. Turning on at the 1.5-fold stage, *clec-56* displays spatial localization within the mid intestine and is sequestered to four intestinal cells. This preliminary data advances our current understanding of intestinal physiology within *C. elegans* and begins bridging a critical knowledge gap in the field. Moreover, it sheds light on if and how critical functions are sub-functionalized throughout the intestine and spatiotemporally regulated over developmental time.

1100F **Assessment of heterogeneity within the *Drosophila* germline stem cell niche** Jennifer Viveiros, Ambarisha Samantaray, Erika Matunis Cell Biology, Johns Hopkins School of Medicine

Adult stem cells reside in dynamic, supportive microenvironments termed niches, which are generated by specialized niche cells. Niches are often complex and composed of functionally cooperative subpopulations of cells rather than uniform populations. Gaining insight into niche composition is fundamental to our understanding of tissue homeostasis. Using the *Drosophila melanogaster* testis stem cell niche as a model, we can further our understanding of stem cell niches by investigating their cellular composition and gene expression programs. This niche contains three cell populations: post-mitotic somatic hub cells (or niche cells), which are surrounded by two types of stem cells, germline stem cells (GSCs) that differentiate into sperm, and cyst stem cells (CySCs) that give rise to somatic support cells. Previous *in situ* hybridization (ISH) and immunostaining experiments have suggested that hub cells may not uniformly express the same transcriptional program, raising the possibility that they are a heterogeneous population of cells. Intriguingly, hub cells descend from somatic gonadal precursors (SGPs), which arise from three distinct parasegments (PS) in embryogenesis, suggesting that developmental origin could underly previously observed heterogeneity. Here, we examine hub cell heterogeneity with respect to function, origin, and position. We observe that not all hub cells require expression of proteins integral to anchoring the hub to the basement membrane but loss of these proteins in the entire hub alone displaces the cluster of cells. Using lineage tracing tools, we find that adult hub cells arising from PS 11 do not differ in position or signaling compared to their counterparts in unperturbed testes. These findings will begin to determine if hub cells are functionally distinct and whether developmental origin correlates with function, localization within the niche, and transcriptional program.

1101F **The *Drosophila* ovary as a model to identify transcriptional targets of the Frazzled receptor** Rhegan M. Murray[1], Kierra P. Parsons[1], Camila M. Barrios-Camacho[2], Greg J. Bashaw[2], Kaitlin M. Laws[1] [1]Biology, Randolph-Macon College, [2]Neuroscience, University of Pennsylvania

In the fruit fly *Drosophila melanogaster,* quality checkpoints during oogenesis prevent female energy investment in low-quality oocytes. For example, either aberrant development or suboptimal nutritional status can activate the mid-oogenesis checkpoint, leading to germ cell degeneration through apoptosis. We previously reported that the axon guidance receptor Frazzled [Fra; Deleted in colorectal cancer (Dcc) in vertebrates] regulates the mid-oogenesis checkpoint. Specifically, germline loss of Fra leads to germline apoptosis at mid-oogenesis; however, how Fra controls oocyte development remains unclear.

Previous investigations of Fra/Dcc signaling focused on the nervous system, where Fra responds to its Netrin ligands to regulate axon guidance and circuit formation. In *Drosophila* commissural neurons, Fra also acts independently of Netrins to regulate gene expression through the transcriptional activity of its intracellular domain. While several players in this Netrin-independent pathway have been identified, only one transcriptional target of Fra is known. Although Netrins are expressed in the adult ovary, *netrin* mutants do not activate the mid-oogenesis checkpoint, and oocytes mature normally. Instead, germ cells require the transcriptional activity of Fra to develop into mature oocytes.

Our central hypothesis is that the transcriptional activity of Fra controls expression of genes that regulate oocyte quality. We expected that transcriptional targets of *frazzled* would also be expressed in the germline and required for progression through mid-oogenesis. To identify these transcripts, we sequenced the transcriptomes of ovaries with and without germline Fra. We identified 177 differentially expressed genes, 155 of which are reported to be expressed in the wild-type ovarian germline. To validate candidate gene expression, we are using publicly available genetic reagents, including gene and enhancer traps. We are concurrently investigating the role of these candidates in the ovary using genetic analyses with both behavioral and morphological readouts. For example, we are using transgenic RNAi lines to reduce candidate gene expression specifically in the fly germline. If the candidate, like Fra, promotes germline survival, we expect these flies to lay fewer eggs than age-matched controls and, upon dissection, to observe cell death at mid-oogenesis. Follow-up experiments will determine whether observed phenotypes are Fra-dependent. Together, these studies will serve as a model for future investigations of guidance receptor signaling in the *Drosophila* ovary and invite comparisons to mechanisms used in the nervous system.

1102F **Bruno1 Isoforms have distinct functions during IFM Myogenesis in *D. melanogaster*** Jenna DeCata[1], Elena

Nikonova[2], Thomas Harr[2], Danei Smith[1], Maria L. Spletter[1] [1]School of Science and Engineering, University of Missouri- Kansas City, [2]Physiological Chemistry, Ludwig-Maximilians-Universitat Munchen

Different muscle fiber types have distinct morphologies and contractile abilities that are established during development by the expression of fiber-type specific isoforms of structural proteins. Developmental and fiber-type specific patterns of isoform expression are regulated through alternative splicing. RNA-binding proteins (RBPs) including members of the conserved CELF (Bruno) family regulate alternative splicing in muscle. Our previous work demonstrated that Bruno1 (Bru1) is required for indirect flight muscle (IFMs) development in *D. melanogaster*, and we recently uncovered a requirement for Bru1 both in early muscle development and later during maturation of the sarcomeres. Bru1 regulates a developmental switch in IFM-specific sarcomere gene isoform expression. Curiously, Bru1 is itself alternatively spliced, but specific functions of the long (RB), middle (RA), and short (RD) isoforms are currently unknown. To test if different Bru1 isoforms have distinct roles in myogenesis, Gal4-UAS was used to control when and where Bru1 isoforms are expressed. Him-Gal4 and Fln-Gal4 were used to drive early and late overexpression of the different Bru1 isoforms. Functional tests and confocal microscopy of adult IFMs revealed distinct roles for Bru1 isoforms in myogenesis. Bru1-RB overexpression resulted in muscle detachment and tubular-like fiber morphology. Bru1-RA overexpression was dosage sensitive and disrupted the actin cytoskeleton causing zebra-bodies in sarcomeres. Bru1-RD overexpression resulted in only minor phenotypes in muscle tissue. There is also a difference in the localization of these isoforms in the IFMs, with some isoforms localized to the nucleus and others cytosolic. These findings demonstrate that the correct isoform as well as the correct expression level of Bru1 isoforms are required for proper IFM formation and function. Our results provide novel insight into mechanisms of RNA regulation during development, potentially identifying a conserved mechanism to fine-tune CELF activity in response to cell identity and developmental status.

1103F **A Conserved Cardiomyocyte-Specific Endocycle Program Underlies Heart Development** Archan Chakraborty[1], Meera Gangasani[1], Alexandra Purdy[2], Dawn Bowles[1], Matthew Wolf[3], Michaela Patterson[2,2], Don Fox[1] [1]Pharmacology and Cancer Biology, Duke University, [2]Medical College of Wisconsin, [3]University of Virginia

During adolescence, mammalian cardiomyocytes lose their ability to proliferate and instead transition to growth through whole genome duplication. A common mechanism of whole genome duplication is the endocycle, which repeatedly replicates the genome to produce a polyploid cell. Unlike many polyploid tissues, the heart has limited flexibility with regards to the upper and lower limit of ploidy needed for optimal function and development. As evidence of this limited flexibility of polyploidy, we discovered that both *Drosophila* and human cardiac organs undergo chamber-specific polyploidization. Manipulating this chamber-specific ploidy asymmetry in *Drosophila* impacts the proper functioning of the fly's cardiac organ, mimicking cardiomyopathies. Therefore, identifying the regulators of cardiac-specific polyploidization is important. To further understand the regulation of cardiac polyploidy we performed a genetic screen using RNA interference (RNAi) in the *Drosophila* larval heart. Leveraging publicly available human cardiomyocyte RNA-Seq data and GWAS data for cardiac ploidy in rats, we targeted 128 ortholog genes in *Drosophila* larval hearts. We identified several classes of genes that regulate cardiac ploidy including chamber-specific polyploidy. Additionally, we identified evidence of a cardiac specific endocycle program. Notably, COX7A, a component of mitochondrial respiratory complex IV showed opposing effects in heart vs salivary gland, another polyploid tissue. In summary, through a genetic screen we have identified conserved cardiac-expressed genes with specific roles in regulating ploidy of heart tissue. This work reveals heart-specific mitochondrial regulation of polyploidy that is distinct from general endocycle regulation.

1104F **Exploring the impact of germ granule diversity on germline development in *Drosophila*** Gisselle A Hidalgo[1], Ahad L Shabazz-Henry[1], Bianca M Ortega[1], Suneth Tissera[1], David A Joiner[1], Matthew G Niepielko[1,2] [1]School of Integrative Science and Technology, Kean University, [2]Department of Biological Sciences, Kean University

The development and maintenance of the germline, the set of highly specialized cells responsible for passing on genetic material to the following generation, is essential for animal reproduction. Germline function and maintenance require the formation of highly conserved ribonucleoprotein (RNP) granules called germ granules. Germ granules are biomolecular condensates that contain many types of mRNAs and proteins that have important roles in germline differentiation, proliferation, and maintaining transcriptional quiescence through post-transcriptional gene regulation. In animals including *Drosophila*, *Xenopus*, and zebrafish, primordial germ cell specification relies on the inheritance of germ granules that reside at a specific location within the egg. Despite the conserved function of germ granules and their components, how quantitative changes in germ granule mRNA content influences germline development remains unclear. We hypothesize that changes in germ granule mRNA content leads to differences in germline development and reproductive health. To test our hypothesis, we analyzed the natural diversity of germ granule mRNA content and its relationship to differences in germline development that occurs in several *Drosophila* species. Using a combination of single molecule fluorescence *in situ* hybridization (smFISH), computational modeling, and immunofluorescence, our preliminary results suggest that changes in germ granule mRNA content leads to an increased presence of defective primordial germ cells in the *Drosophila* embryo.

Together, our findings demonstrate how germline development relies on genetic mechanisms that have the capacity to fine-tune germ granule mRNA content.

1105F **Regulation of Stem Cell Lineage Behavior in *Drosophila* Testes by the Novel Gene, *CG11180/Chigno*** Claire Aminuddin, Rina Shou, Natalie Luffman, Morgan Claybrook, Oliver Kerscher, Matthew Wawersik Biology Department, College of William and Mary

Stem cells are critical for organ function and tissue homeostasis. Through asymmetric division, they produce two daughter cells – one that retains stem cell fate, and one that differentiates according to organ function. Stem cells can also divide symmetrically to restore or amplify the stem cell population. *Drosophila* testes are a key model system for understanding molecular mechanisms regulating stem cell maintenance and differentiation. This system consists of two stem cell types: sperm-producing germline stem cells (GSCs) and somatic cyst stem cells (CySCs), which maintain the GSC population and produce cyst cells that nurture spermatogenesis. These two stem cell types are docked at a cluster of somatic hub cells that reside at the testis apex. Work from the Wawersik lab has identified the novel gene, *CG11180/Chigno*, as a potential regulator of CySC maintenance and differentiation (Rinhart et al, 2021). Somatic knockdown of Chigno in the CySC lineage causes an expansion of under-differentiated germ cells and defects in spermatogenesis that lead to infertility. Here we further explore the function of Chigno in *Drosophila* testes. Specifically, we examined Chigno expression, sub-cellular localization, and the impact of Chigno RNAi knockdown on CySC maintenance and differentiation. Data indicate that Chigno is localized to the nucleolus of GSCs, CySCs, and their early progeny in adult testes, with low expression in hub cells. We also find that knockdown of Chigno in the CySC lineage causes CySC expansion and defects in cyst differentiation. Interestingly, an expansion of hub cells is also observed. Ongoing experiments examine mechanisms by which these defects occur, and potential roles of Chigno in the germline. As Chigno shares homology with PINX1, an RNA binding protein and telomerase inhibitor expressed in mammalian reproductive tissues, these data have important implications for human infertility and reproductive cancer.

1106F **Controlling BMP Pathway with Optogenetic Receptors in Drosophila Embryos and Germline Stem Cells Niche** Hung-Yuan (Zeke) Chen, Gregory Reeves Chemical Engineering, Texas A&M University

The Bone morphogenetic protein (BMP) signaling pathway is highly conserved and regulates several cellular processes across the animal kingdom, such as tissue patterning and stem cell maintenance. In Drosophila, the BMP homolog Dpp activates the Type I receptor, Thickveins (Tkv), and the Type II receptor, Punt (Put), and the activated receptor complex then phosphorylates the Smad1 homolog Mothers against Dpp (Mad). Phosphorylated Mad (pMad) then binds to the cofactor Medea (Smad4 homolog) and the complex translocates to the nucleus to regulate BMP target genes. Here we performed live imaging with fluorescently-tagged Mad and Medea in the early Drosophila embryo (2-3 h old) and in female germline stem cells (GSCs). We quantified the dynamics of the BMP gradient and used raster image correlation spectroscopy (RICS), a derivative of fluorescence correlation spectroscopy, allowing us to visualize their absolute concentration and the extent to which they are in a complex with each other. We also used the optogenetic LOV2 system to photoactivate the Type I and Type II BMP receptors. This opto-receptor system forms dimers when exposed to blue light, leading to phosphorylation of Mad and initiation of the pathway without Dpp. We tested these constructs in Schneider 2 cells and quantified BMP dynamics in cell culture. These methods have allowed us to probe the longstanding question of how signaling dynamics regulates gene expression on a fundamental level.

1107F **Myosin II is required for seamless tubulogenesis and unicellular branching morphogenesis in *Drosophila* tracheal terminal cells** Jodi Schottenfeld-Roames[1], Tanner Simpson[1], Ivan Ruiz Leon[2], Kaelix Johnson[2] [1]Molecular Biology, Princeton University, [2]Princeton University

Transformation of epithelial cells into complex functional organs is a critical step in development. Organs such as the lungs and kidney are composed of multicellular tubes (two or more cells attached to one other by junctions), whereas a large percentage of capillaries in the vertebrate vascular system are composed of seamless tubes (single cells that lack junctions). Although the morphogenetic events that help generate a multicellular tube can vary, it is clear how cells joined together by epithelial junctions create a membrane-bounded lumen; what is not obvious is how a single cell hollows out to create an internal lumenal membrane *de novo*. Specialized tips cells in the *Drosophila* tracheal system called “terminal cells” go on to form branched seamless tubes. Using terminal cells as a model, we have uncovered a number of cytoskeletal and membrane trafficking regulators that help orchestrate this seamless cell-to-tube transformation in previous work. Candidate RNAi screens to identify additional factors required for terminal cells morphogenesis have served as the foundation for an upper-level undergraduate research course. One such protein identified by students in the course is the actin contractibility regulator, Myosin II. Here, we show a novel role for Myosin II during the process of seamless tubulogenesis and unicellular branching morphogenesis the fly tracheal system. RNAi against the fly *myosin II regulatory light chain* (*spaghetti-squash)* and *myosin II heavy chain* (*zipper)* results in changes to terminal cell architecture and discoordination between branch and lumen formation.

A student-developed method for analyzing branch density within defined quadrants of space around a designated plane of a terminal cell reveals that Myosin II depleted cells have reduced spreading compared to wild type control cells. Efforts to determine the mechanism by which Myosin II regulates branching and tube formation in terminal cells are currently underway.

1108F **Fox transcription factor-mediated morphogenesis of the alary muscles associated with the *Drosophila* heart** Kuncha Shashidhar, Rajnandani Katariya, Md. Rezaul Hasan, Mofazzal K Sabbir, Abbigayle J Gamble, Shaad M Ahmad Biology, Indiana State University

Eight Forkhead box (Fox) transcription factors (TFs) are required for proper cardiogenesis in mammals while mutations in four Forkhead box (Fox) genes have been linked to human congenital heart disease. Our prior work had identified the conserved roles of two *Drosophila* Fox genes, *jumeau* (*jumu*) and *Checkpoint suppressor 1-like* (*CHES-1-like*) in mediating cardiac progenitor cell specification, division, differentiation, and positioning. Here we describe an additional role of the *Drosophila* Fox gene *CHES-1-like* in mediating the development of alary muscles (AMs), structures which connect the heart to the embryonic/larval exoskeleton and are essential for supporting the heart, stabilizing its location, maintaining its lumen, and controlling ostia opening and heart beating. In *CHES-1-like* loss-of-function mutants, one or more of the fourteen AMs per embryo exhibit significant morphological defects, being deformed, truncated, or missing. One or more of the following hypotheses could explain these defects in AM morophogenesis: (1) incorrect specification of AM founder cells, (2) defective myoblast fusion, (3) flawed myotube elongation, (4) incorrectly positioned AM attachment sites on the heart due to cardiac progenitor cell division errors, and (5) improper muscle attachment site selection. We are in the process of conducting experiments to determine which of these hypotheses are correct and thereby to begin exploring the mechanism by which this Fox gene mediates development of associated tissues supporting the heart.

1109F ***Drosophila pseudoobscura* sperm heteromorphism: Genetic and cellular processes regulating sperm length** Fiona Messer[1], Helen White-Cooper[2] [1]Biosciences, Cardiff University, [2]Cardiff University

Drosophila pseudoobscura produce three distinct sperm morphs: a long fertilising morph, the eusperm, and short and medium non-fertilising morphs, parasperm 1 and 2. Parasperm protect the eusperm from female-derived spermicides in the female reproductive tract. The three morphs are produced simultaneously and consistently throughout the lifetime of the fly. *D. pseudoobscura* is therefore an ideal system to investigate the genetic and cellular processes regulating sperm length.

Drosophila spermatogenesis follows a well-characterised pattern of differentiation, mitosis, meiosis, elongation, and individualisation. Transcription of genes whose products are required during meiosis and post-meiosis predominantly occurs during the pre-meiotic primary spermatocyte stage. Our previous work has identified transcriptional differences between sub-sets of cysts, prior to the onset of meiosis, including components of the testis meiotic arrest complex (tMAC) and the tMAC regulator *kumgang (kmg)*. We propose that these transcriptional differences underpin sperm morph differentiation. GFP-tagged Kmg protein is also differentially expressed in *D. pseudoobscura* spermatocytes, and cell culture establishes morph identity of high, medium, and low Kmg-expressing cysts.

HCR-FISH screening of the cup- and comet-class genes has also revealed post-meiotic differential gene expression and localisation between elongating spermatid morphs. Some of these post-meiotically expressed genes are transcribed and localise to the growing tail ends of all sperm morph cysts (*f-cup, sunz2B, stress response protein NST1*), however others localise to a sub-set of cysts, either by developmental stage (*schuy*) or by morph (*wa-cup, sunz2A, sunz3*).

Whole testis single-cell RNA sequencing is used to identify the transcriptional profiles of each sperm morph throughout spermatogenesis, in addition to the somatic cyst cell profiles. Trajectory analysis can also reveal the timing of morph differentiation, early differentially expressed genes and potential downstream targets of previously identified differentially expressed TMAC components and Kmg.

1110F **Distinct roles for COMPASS core subunits Set1, Trx, and Trr in the epigenetic regulation of *Drosophila* heart development** Junyi Zhu, Joyce van de Leemput, Zhe Han University of Maryland Baltimore

Histone methylation regulates transcriptome dynamics during development and aging, as well as in disease. Various methyltransferases have been linked to heart disease, through disrupted expression and activity, and genetic variants associated with congenital heart disease. However, in vivo functional data for many of the methyltransferases in the context of the heart are limited. Here, we used the *Drosophila* model system to investigate different histone 3 lysine 4 (H3K4) methyltransferases for their role in heart development. A series of multiprotein complexes termed Complex of Proteins Associated with Set1 (COMPASS) are involved in H3K4 methylation. *Drosophila* Set1, trx and trr formed the core subunits of these complexes. Development in flies deficient for these three core subunits was notable for the high lethality at eclosion and significantly shortened lifespans for adults that did emerge. Silencing of *Set1*, *trx,* and *trr* in the heart led to a reduction

of H3K4 monomethylation (H3K4me1) and dimethylation (H3K4me2) differently indicating their different roles in H3K4 methylation. Furthermore, we identified the gene expression patterns regulated by *Set1*, *trx,* and *trr*. These data suggest each of the COMPASS core subunits controls methylation of different sets of genes, with many metabolic pathways on early in development and throughout, while muscle and heart differentiation processes are methylated during later stages of development. Taken together, our findings demonstrated the roles of COMPASS series complex core subunit Set1, trx, and trr in regulating histone methylation during heart development, with implications for CHD.

1111F **Dissecting the temporal dynamics of histone inheritance through *Drosophila* neural development** Jason T Palladino[1], Xin Chen[1,2] [1]Biology, Johns Hopkins University, [2]Howard Hughes Medical Institute

Proper development depends on asymmetric cell division (ACD), a process by which dividing stem cells produce a renewed stem cell and a differentiating cell. Many intrinsic and extrinsic factors guiding ACD have been found, however, the role of chromatin to ACD is poorly understood. Previously, our lab discovered asymmetries in histone and histone post-translational modification (hPTM) inheritance in *Drosophila* male germline stem cells (mGSCs). Disruption of these asymmetries results in both stem cell loss and overpopulation of progenitor cells phenotypes, suggesting that asymmetric histone inheritance is an essential process in tissue health. Further, deterioration of this process may be common among diseases including tissue degeneration and cancer. Investigation into other adult stem cell lineages showed asymmetric histone inheritance, suggesting it is a general mechanism. However, whether histone inheritance patterns influence cell-fate decisions for more potent developmental stem cells remains to be determined.

To interrogate the role of histone inheritance in development, I use *Drosophila* neural stem cells, neuroblasts (NBs), a well-studied model for ACD. Similar to mGSCs, inheritance of total H3 and H4 in NBs is also asymmetric, suggesting histone density asymmetry is conserved between mGSCs and NBs. Despite commonalities, histone inheritance in NBs is distinct from mGSCs in several ways. First, the polarity of histone asymmetry in NB ACD is extremely dynamic with all possible patterns being observed. Second, the strength of asymmetric histone inheritance increases with developmental age with older NBs displaying a higher degree of histone asymmetry. As NB progeny identity is also dynamic and dictated by molecular clocks, I hypothesize inheritance of histones and associated epigenetic information contributes to cell-fate determination in NB lineages. Indeed, tracking of the Polycomb-associated hPTM H3K27me3 using an eGFP-tagged mintbody shows a high positive correlation between H3K27me3 and total H3 inheritance during NB ACD. Given that H3K27me3 is enriched with old histones, this positive correlation indicates that old H3-enriched sister chromatids have higher histone density, consistent with the results we have obtained in mGSCs. Further manipulation of NB identity and epigenetic regulators will elucidate the contribution of epigenetic inheritance to complex cell-fate decisions during development, which I will discuss further.

1112F **Deciphering the function of CNK during thorax closure in Drosophila** Eloïse Duramé[1,2], Caroline Baril[1,2], Malha Sahmi[1,2], Marc Therrien[1,2] [1]Université de Montréal, [2]IRIC

Scaffold proteins enable selectivity and precise spatiotemporal regulation of signal transduction. They achieve this by assembling molecular complexes and directing them to particular subcellular sites. Connector eNhancer of Kinase suppressor of RAS (CNK) family members serve as scaffolds that have been conserved throughout evolution. CNK was initially identified in Drosophila, where it acts as a positive regulator of the receptor tyrosine kinase-induced RAS/MAPK pathway. Although the exact role of CNK family proteins is not fully understood, our recent findings highlight CNK2A's importance in promoting human cancer cell migration by activating RAC1 and inhibiting RHOA through ARF6 activation. This led us to explore the role of CNK during cell migration in Drosophila.

In this study, we used thorax closure as a model to study migration. During pupal thorax morphogenesis, the dorsal epithelium of the two imaginal wing discs migrate over an underlying larval epidermis and fuses at the midline to form a continuous thorax. Interestingly, knockdown of *cnk* during thorax morphogenesis using the pannier-Gal4 driver led to thorax closure defects. Time-lapse imaging of thorax closure showed that *cnk* depletion disrupts larval epidermis integrity, reduces thoracic cell velocity and prevents the fusion of the migrating discs. Overexpressing the C-terminal part of CNK, but not the N-terminal part, produced the same defect as *cnk* depletion. Notably, CNK›s role during thorax closure appears to be independent of its function in the RAS-MAPK pathway but relies on its localization to the plasma membrane through its PH domain. To gain mechanistic insights into the function of CNK in this biological event, we used the miniTurboID technique to identify proximal CNK interactors during thorax closure. This approach uncovered interesting candidate interactors, including Par-1 and subunits of the PP2A complex. We are currently assessing these candidates in the context of thorax closure and their functional connections with CNK through genetic interaction experiments.

Together, our results strongly suggest a role for CNK during thorax morphogenesis in Drosophila, and further work should refine our understanding of the molecular mechanisms underlying epithelial migration. Additionally, it will shed light on the

role of CNK in signaling contexts beyond the RAS-MAPK pathway.

1113F **Investigation of gene functions in secretory granule formation in *Drosophila* salivary glands** Navid Tahan Zadeh, Yevin Chung, Liping Zhang, Kelly Ten Hagen, Olivia Fish, Robin Black, Amy-Claire Dauphin National Institute of Health

Regulated secretion is an essential process by which proteins, stored in secretory granules, are released into the extracellular environment in response to some stimulus. Defects in this process, can contribute to numerous diseases, including cystic fibrosis and inflammatory bowel disease. In *Drosophila* larval salivary glands, large and highly glycosylated proteins, such as mucins, are produced, packaged, and released in response to developmentally regulated pulses of the hormone, 20-hydroxyecdysone. To understand this highly orchestrated process and identify the factors responsible for mucin biosynthesis and secretory granule maturation, we performed single-cell RNA sequencing on *Drosophila* salivary glands and revealed the presence of five secretory cell-like clusters. To further identify genes involved in mucin synthesis, packaging, and secretion, we performed RNAi on the genes identified from secretory cell clusters. Here we show that two genes, *CG3168* and *Gale* are involved in secretory granule formation. In the future, deeper analysis of the scRNA-seq data and further investigation of genes identified in each secretory cell clusters will allow for a better understanding of the secretory granule biogenesis and regulated secretion.

1114F **Invagination in the *Drosophila* Salivary Gland is Mediated by Huckebein Through Regulated Secretion of the GPCR-Pathway Ligand Folded gastrulation (Fog)** Ashleigh M Shoemaker[1], Ji Hoon Kim[1], Anuradha Ratnaparkhi[2], Deborah J Andrew[1] [1]Cell Biology, Johns Hopkins School of Medicine, [2]Developmental Biology, Agharkar Research Institute

Drosophila melanogaster is a valuable model organism for revealing the cellular and molecular basis of congenital diseases. Our laboratory utilizes the embryonic *Drosophila* salivary gland (SG) as a simple model of epithelial tube formation analogous to the mammalian lungs and glandular tissues. While the cellular mechanisms contributing to SG invagination have been described, the signaling pathways coordinating this process remain unclear. We hypothesize that the SP1-like transcription factor Huckebein (Hkb) mediates the localized secretion of the signaling molecule encoded by Folded gastrulation (Fog), thus activating the downstream G-protein-coupled-receptors (GPCRs) critical for cellular invagination. Fog and/or its GPCR may then be recycled to the apical membrane by vesicles expressing the kinesin-binding protein Klarsicht, whose transcription is *hkb*-dependent. Utilizing targeted gene disruption of *hkb* by CRISPR/Cas9 genetic engineering, in combination with tissue-specific misexpression of *hkb*, I test the model that Hkb regulates secretion of Fog. Our work reveals that high levels of Fog secretion is limited to the SG cells about to invaginate, and that misexpression of Hkb in all SG cells affects the levels and distribution of secreted Fog. Preliminary data suggests that changes in Hkb expression result in aberrant invagination. Next, we will determine the localization of Fog and its corresponding GPCR in the absence of *hkb* and Hkb downstream transcriptional targets linked to vesicle trafficking. Our findings suggest that Hkb facilitates the localized secretion of Fog from SG cells, which then activates the downstream GPCR-mediated pathway required for the cell shape changes that underlie invagination.

1115F **Dissecting Fox transcription factor-mediated regulation of Polo kinase activity essential for cardiac progenitor cell divisions** Rajnandani Katariya[1,2,3], Abbigayle J. Gamble[1,2], M. Rezaul Hasan[1,2,3], Kuncha Shashidhar[1,2], Mofazzal K. Sabbir[1,2], Shaad M. Ahmad[1,2,3] [1]Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center (PCRC), Indiana State University

Forkhead box (Fox) transcription factors are critical for cardiogenesis in both mammals and *Drosophila*. Previous work from our laboratory has shown that the *Drosophila* Fox gene *jumeau* (*jumu*) mediates three distinct categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage—by upregulating Polo kinase activity. However, a comparison of transcription expression profiles of flow cytometry-sorted mesodermal cells from wild-type embryos and *jumu* null mutants showed that *jumu* does not transcriptionally regulate *polo*, thereby suggesting that the Fox-mediated activation of *polo* for cardiac progenitor cell division may be posttranscriptional. A known crucial step in the post-transcriptional activation of *polo* is the post-translational phosphorylation of Polo protein. Two serine-threonine kinases that activate Polo protein via phosphorylation are Aurora B (AurB) and Back seat driver (BSD). Intriguingly, *aurB* and *bsd*, the genes encoding these kinases, are both transcriptionally activated by *jumu*, making these kinases promising candidates through which the Fox gene may activate *polo. aurB* is already known to play multiple roles in mitosis. And while *bsd* has previously been characterized only as mediating the post-mitotic activation of Polo necessary for somatic muscle morphogenesis, it does not preclude *bsd* from also being utilized for mitosis in cardiac progenitor cells. If *jumu* does indeed utilize *aurB* and *bsd* to activate *polo* for cardiac progenitor cell division, then we would expect *aurB* and *bsd* mutants to exhibit cardiac defects similar to those in *jumu* and *polo*mutants. Here we show that loss-of-function mutations of *aurB* and *bsd* do indeed phenocopy all three categories of cardiac progenitor cell division defects observed in *jumu* and *polo* mutants, consistent with such a role. We are presently utilizing genetic interaction and rescue assays to conclusively determine whether *aurB* and *bsd* mediate Polo activation by *jumu*. If *jumu* does indeed activate Polo using one of these two candidate genes, then that candidate gene should

exhibit pairwise synergistic genetic interactions with both *jumu* and *polo*. Furthermore, independent cardiac-specific ectopic expression of the candidate gene should partially rescue the cardiac progenitor cell division defects of *jumu* mutants, but not of *polo* mutants.

1116F **Shocking development: Heat shock factor functions through Hsp83 to regulate *Drosophila* development** Noah Reger[1], Jinghong Tang[2], Somes Schwinghammer[2], Michael Welte[2] [1]Biology, University of Rochester, [2]University of Rochester

Heat shock factors (HSFs) are a family of conserved transcription factors well-known for their roles in regulating stress responses. Here, HSFs largely act through upregulation of Heat Shock Proteins (HSPs) which function as molecular chaperones to facilitate proper protein folding post-stress. Compared to this canonical role of HSFs, their non-canonical roles have garnered relatively little attention. HSF functions in the regulation of early development in *C. elegans* and is required for proper post-natal growth in mice. Mammalian HSF1 is also necessary for robust growth of almost all tumors. The targets of HSF in these developmental contexts are either known or suspected to be proteins not typically upregulated upon stress. Here we interrogate the developmental role of the single HSF family member in *Drosophila*. Because larvae lacking HSF die early during development, we selectively knocked down HSF in several larval tissues that undergo massive growth. Knockdown in the prothoracic gland (PG) resulted in a reduction of both cell size and DNA content. Similar results were observed in the salivary glands—another endoreplicative tissue. Knockdown in wing discs lead to malformed adult wings. These results strongly indicate that HSF functions broadly in *Drosophila* development. Additionally, we observed that PG knockdown of HSF caused larvae to arrest during the third instar stage. The PG secretes the hormone ecdysone necessary for the juvenile-to-adult transition in flies. Ecdysone feeding was sufficient to rescue larval arrest indicating that these animals fail to generate sufficient ecdysone. Previous work had shown that under non-stress conditions HSF enhances Hsp83 expression in cultured cells and salivary glands; Hsp83 is the fly version of Hsp90. We find that the PG highly expresses Hsp83 during normal development and expression is lost in an HSF deficient background. Overexpressing Hsp83 in such a background was sufficient to fully rescue the PG phenotypes as well as larval arrest, indicating HSF functions through Hsp83 to promote PG development. Hsp83 overexpression also partially rescues the defects observed in salivary glands and wing discs lacking HSF. Future experiments will focus on determining whether Hsp83 regulation explains all of HSF's developmental roles or whether HSF works in addition with the ecdysone signaling pathway.

1117F **Rho kinase regulates spermatogenesis in Drosophila germline** Carihann M Dominicci-Cotto[1], Josefa Steinhauer[2], Andreas Jenny[3,4] [1]Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]Biology, Yeshiva University, [3]Develomental and Molecular Biology, Albert Einstein College of Medicine, [4]Genetics, Albert Einstein College of Medicine

A higher species' survival requires proper gamete production and formation. Sperm count in men has decreased significantly over the last 50 years. Therefore, understanding the signaling pathways that regulate sperm production and fertility is crucial for humanity's survival. During spermatogenesis in *Drosophila melanogaster*, syncytial spermatids differentiate and individualize into mature sperm tightly enclosed by a plasma membrane. Specialized actin cones move along the sperm tails to separate spermatids during individualization. In this process, inter-spermatid bridges and the excess of the cytoplasm are removed, a prerequisite for sister spermatids to become individual, functional spermatids.

Our laboratory previously showed that Combover (Cmb), a member of the family of intrinsically disordered proteins and an effector of planar cell polarity under the control of Rho kinase (Rok), is essential for sperm individualization and fertility in Drosophila. We demonstrated that the individualization defects present in the *cmb* mutant can be rescued by the expression of wild-type Cmb. Intriguingly, the rescue depends on the presence of WT Rok phosphorylation sites in Cmb, suggesting that Cmb function on sperm individualization requires Rok. Here, we show that loss of Rok in the germline, using a CRISPR-generated *rok* mutant (Crispant), causes infertility in adult male flies. To determine the cause, we measured the length of the testis and quantified the individualization defects. We found that Rok decrease causes a reduction in testis length and an increase in defects of individualization complexes. Furthermore, we find that endogenously tagged GFP-Cmb is enriched in the axoneme elongation complexes (ECs), which show a reduced αSpectrin staining in *rok* Crispants. We, therefore, show for the first time that Rok and Cmb are required in the male germline and hypothesize that they regulate a transition between elongation and individualization of spermatids.

1118F **Fox transcription factors mediate proper positioning of cardiac cells by restricting the expression of ECM genes** Rajnandani Katariya[1,2,3], Manoj Panta[1,2], Andrew J. Kump[1,4], Neal Jeffries[5], Shaad M. Ahmad[1,2,3] [1]Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center (PCRC), Indiana State University, [4]The Center for Genetic Advocacy, Indiana State University, [5]Office of Biostatistics Research, National Heart, Lung and Blood Institute, NIH

The development of a complex organ requires the specification of appropriate numbers of each of its constituent cell types as well as their correct positioning of these cell types within the organ. We previously showed that the Fox transcription factors (TFs) Checkpoint suppressor homologue (CHES-1-like) and Jumeau (Jumu) determine the correct number of different cardiac cell types by regulating cardiac progenitor cell divisions. Here we show that *CHES-1-like* and *jumu* are also required for the correct positioning of these cardiac cell types: null mutations in either gene result in the misalignment and incorrect location of cardial and pericardial cells within individual hemisegments. Since defective cardiac progenitor cell divisions in *CHES-1-like* and *jumu* loss-of-function mutants frequently result in individual hemisegments having different numbers of cardiac cells than their partners across the dorsal midline, we first examined this asymmetry as a possible steric cause of incorrect positioning. Our analysis revealed that steric constraints imposed by the differing number of heart cells in contralateral hemisgments cannot explain all of the observed defects in cardiac cell positioning: statistically significant increases in the number of positioning defects are also observed in Fox mutants compared with wild-type embryos when only members of contralateral hemisegment pairs having the same number of each cardiac cell type are compared. In order to discover the other cause underlying positioning defects, we compared genome-wide transcription expression profiles of purified mesodermal cells from wild-type embryos and embryos lacking functional copies of *CHES-1-like*, *jumu*, or both Fox genes to identify Fox-regulated targets. Among the 2,131 target genes we identified, genes encoding extracellular matrix (ECM) proteins were overrepresented among genes repressed by the Fox TFs. In particular, the ECM proteins Viking, Collagen type IV alpha 1, and Terribly reduced optic lobes were all overexpressed in Fox mutants. Our preliminary phenotypic analysis of these specific targets suggests that the Fox TFs bring about the correct positioning of cardiac cell types by restricting their expression: independent ectopic overexpression of each of these ECM genes in the mesoderm phenocopies the cardiac cell positioning defects observed in *CHES-1-like* and *jumu* loss-of-function mutants.

1119F **The Role of Drop on Salivary Gland Morphogenesis** Matthew Elliott[1], Deborah Andrew[2] [1]Cell Biology, Johns Hopkins University, [2]Cell Biology, Johns Hopkins School of Medicine

Recently, single cell RNA sequencing (scRNA-seq) data from our lab revealed transient expression of *Drop* (*Dr*) mRNA in the salivary gland (SG) during early organ development. *Dr* is commonly known for its dominant gain-of-function mutation, which results in the small eye phenotype that is used as genetic marker for easy identification in crosses with the 3rd chromosome. *Dr* encodes for a homeodomain transcription factor most closely to the human Msx1 and Msx2 proteins. Previous data implicates *Dr* in the specification of myoblasts and neuroblasts, development of muscle and neuronal cells, and regulation of glucose metabolism. Since *Dr* expression in the SG was unknown, its role in the SG has not yet been elucidated. Our preliminary findings from immunostained embryos from a *Dr* insertion line showed a phenotypical change in SG morphology consistent with abnormal cell invagination. Furthermore, our scRNA-seq data showed *Dr* mRNA expression only in cells that are first to invaginate during SG morphogenesis. Thus, we hypothesize *Dr* may play a role in SG invagination, tube elongation, and/or cell viability. To test this idea, we have developed tagged and untagged Gal4/UAS-expression constructs to drive persistent expression of *Dr* in the entire embryonic SG. We are also developing CRISPR-Cas9 knock-outs of Dr to determine the effects of null mutations in *Dr* on formation of the SG. These tools will allow us to gain a full understanding of the involvement of *Dr* in the SG during morphogenesis. Furthermore, our findings may shed light on the role of its human orthologs in early developmental processes.

1120F ***trithorax (trx)* and *trx* group (*trxG*) regulation of cardiac *Hox* gene expression and patterning of the *Drosophila* dorsal vessel** Sumaiya Islam, Md Sayeed Abu Rayhan, Adam J Farmer, Shaad M Ahmad, Kristopher R Schwab Biological Sciences, Indiana State University

The *trx* and *trxG* genes encode conserved chromatin regulatory proteins that positively control developmental genes, such as the *Homeotic (Hox*) genes, during embryogenesis. In addition to patterning the anterior-posterior embryonic axis, *Hox* genes control the regional patterning of the developing heart in both insects and mammals. However, the precise regulation of cardiac *Hox* expression by these genes has yet to be explored in experimental models of heart development.

In *Drosophila, abdominal-A (abd-A)* expression patterns the posterior embryonic dorsal vessel into the heart proper. This cardiac region is characterized by an enlarged lumen containing valvular ostial cells, which regulate hemolymph flow through the dorsal vessel. We have previously identified that the *trx* is necessary for *abd-A* expression within the posterior dorsal vessel, while *Polycomb (Pc)* represses *abd-A* expansion into the anterior aorta. For example, the loss of *abd-A* expression within the *trx* mutant causes a dramatic homeotic transformation of the posterior heart tube into an anterior aortic-like fate.

The TrxG family of proteins controls the activation and maintenance of gene transcription via the recruitment of multi-protein complexes that perform diverse regulatory functions, including chromatin remodeling, histone methylation, and transcriptional co-activation. Furthermore, Trx regulatory activity is separable into catalytic H3K4 methyltransferase activity and non-catalytic transcriptional coactivation. Therefore, the loss of *abd-A* expression and heart-proper patterning offers a robust assay to further characterize the TrxG control of cardiac *Hox* regulation within cardiogenesis. An allelic series

of *trx* mutant strains that rescue null *trx* embryonic lethality were investigated for *abd-A* expression and heart-proper defects. Interestingly, the trx^{B20} and trx^{B26} homozygous embryos maintained posterior *abd-A* expression and heart-proper patterning. Key components of the Trx Complex Proteins Associated with Set1-like (COMPASS-like) were also investigated for heart defects. Surprisingly, homozygous will die *slowly (wds)* and *absent, small, or homeotic discs 2 (ash2)* mutants possess no significant changes to *abd-A* expression or heart-proper development. Overall, these results suggest diverse *trxG* regulatory activities controlling *Hox* expression and patterning in embryonic heart development.

1121F **Investigating the role of C(2)M dynamics in Cohesin Protein Loading and Formation of the Synaptonemal Complex During meiosis in Female *Drosophila melanogaster*** Margaret Howland[1], Helen Nguyen[2], Kim R McKim[1] [1]Genetics, Rutgers University - New Brunswick, [2]Rutgers University - New Brunswick

Meiosis allows sexually reproducing organisms to reduce their chromosome content in half, which is required for the production of sperm and oocytes. Errors in meiosis can lead to nondisjunction events, aneuploidy, and infertility in humans. Fidelity of homologous chromosome pairing is critical during the first meiotic division to ensure the accuracy of the reductional division. The Synaptonemal Complex (SC) is necessary for chromosome pairing during the formation of bivalents and to initiate DNA double-strand breaks (DSBs), crossover events, and eventual chromosome segregation. There are two meiotic cohesin complexes that facilitate chromosome cohesion and assembly of the SC in Drosophila oocytes. The first complex containing SMC1/SMC3/C(2)M/SA is involved in homologous chromosome pairing while the other complex containing SMC1/SMC3/SOLO/SUNN is required for sister chromatid cohesion. Previous immunofluorescence studies have led to the hypothesis that C(2)M is a kleisin protein that makes a ring structure by binding to both SMC1 and SMC3. How this promotes SC assembly is not known. In support of this hypothesis, we have found that point mutations that would disrupt the proposed C(2)M-SMC interaction domains leads to defects in SC assembly. We have also used heat shock induced expression of SA and SMC, and C(2)M co-expression experiments, to show that the nuclear import and dynamic protein loading of SA and SMC during meiotic prophase depends on C(2)M co-expression. This result indicates that C(2)M may be the limiting factor in dynamic SC formation. It is possible that there are two populations of C(2)M that allow both dynamic and more stable loading throughout prophase I, or that the C(2)M loads flexibly in and out of the complex while the rest of the ring stays on the chromosomes. While C(2)M heat shock induced expression in prophase can rescue a defective SC, future experiments will use RNAi to knockdown C(2)M late in meiotic prophase to determine if prophase loading is required to maintain the SC. Additional future directions include analyzing the role of cohesins in the formation and location of chromatin loops in topologically associating domains and their role in organizing the genome during meiosis. These results will help us understand the role of C(2)M in cohesin protein loading and the formation of the synaptonemal complex.

1122F **A cell death sensitivity and injury resistance switch in long-lived intestinal enterocytes** Jessica Sawyer, Ruth A Montague, Paulo Belato, Olivia Goddard, Don T Fox Department of Pharmacology & Cancer Biology, Duke University

Many metazoan tissues harbor long-lived cells that are not replenished by stem cells. In the absence of stem cells, long-lived cells depend on cellular survival mechanisms. Here, we show that adult *Drosophila* hindgut ileum is a model to uncover mechanisms of tissue longevity and injury resistance. The *Drosophila* hindgut epithelium contains the pylorus, an anterior contractile segment, that moves food along the alimentary canal. The posterior ileum contains large, polyploid enterocytes that are important for salt and water balance. We have previously shown that ectopic expression of pro-apoptotic genes *hid* and/or *rpr* causes tissues injury and cell death, followed by regenerative hypertrophy in the pyloric epithelium. In contrast, we show that expression of *hid* does not injure the adult ileal epithelium, nor does it activate a caspase sensor or cause cell death. This apoptotic insensitivity is specific to *hid*, as ectopic expression of a downstream *hid* target, caspase *dronc* (Caspase-9), injures the adult ileum. Unlike the compensatory hypertrophic regenerative response to cell death in the pylorus after *hid* expression, *dronc*-mediated cell death in the ileum does not activate a robust regenerative response. The *hid*-sensitive pylorus and *hid*-insensitive ileum are derived from the same progenitor population during metamorphosis. *hid*-sensitivity arises during development, as *hid* expression causes injury and cell death in the ileum in young adult animals (less than one day post eclosion). We find that in young animals, both the pylorus and ileum are *hid*-sensitive. Our results suggest that long-lived, non-regenerative intestinal enterocytes of the ileum activate resistance to cellular insults as the adult hindgut matures. To understand the mechanisms of resistance to injury and cell death that arises as the hindgut matures, we isolated ileums from young and mature adults and performed RNAseq. Using these data, we are conducting two candidate screens. First, an RNAi screen of genes elevated in mature ileums. Second, an overexpression screen of genes elevated in young ileums. In both of these screens, we will determine if any of our candidate genes can sensitize the mature ileum to *hid*-mediated injury and cell death. Our results demonstrate that the *Drosophila* hindgut is an excellent model to reveal unique molecular regulation underlying a tradeoff between regeneration and longevity.

1123F **Understanding *Drosophila* neurogenesis at the niche level** Sagar N Kasar, Sarah Siegrist Biology, University of Virginia

Neural stem cells (NSCs) are required to produce the immense diversity of neuron types in the correct time and space for functional neural circuitry. In the developing brain of *Drosophila*, the NSCs, called neuroblasts reside within contained microenvironments called niches, which regulate their behavior by orchestrating intrinsic and extrinsic cues. Neuroblasts are closely associated with glia and thought to form a major structural component of the neurogenic niche. Glia establish an extensive network in the developing brain but little is known about how individual glial subtypes contribute to neuroblast behavior during neurogenesis. We aim to understand how cortex glia contribute to the neurogenic niche. During larval neurogenesis, cortex glia ensheath individual neuroblasts and their newborn progeny to form checkerboard-like cell compartments. We found that the temporal loss of cortex glia causes neuroblast death that leads to the significant reduction in the neuroblast number and smaller sized brains with defects in neuropil structures in surviving adults. We also observed significant structural changes in cortex glial morphology where previously established cortex glial membrane processes retract to form globular glia. We further aim to study the characteristics of such globular cortex glia as well as the signaling crosstalk between neuroblast and cortex glia. This project will help us understand the regulatory role of glial niche during neurogenesis which can provide a novel approach to designing therapeutics for microcephaly and brain tumors.

1124F **Tube dimensional control by apical extracellular matrix modification** James L Woodward[1], Se-Yeon Chung[2] [1]Louisiana State University, [2]Biological Sciences, Louisiana State University

The generation of appropriate tube dimensions during organ formation is critical for organ function and animal homeostasis. Increasing evidence suggests that the apical extracellular matrix (aECM), the extracellular layer on the apical (luminal) side of epithelia, actively contributes to epithelial morphogenesis. In particular, post-translational modifications of aECM components, such as modification of chitin by chitin deacetylases and hydroxylation of collagen by prolyl 4-hydroxylase, are essential for proper tube dimensions. However, very little is known about the structural components and biological functions of aECM, and the mechanisms of tube size control by aECM remain elusive. From our pilot screen in the *Drosophila* embryonic salivary gland (SG) to identify key enzymes that affect tube morphology, we identified Papss, an enzyme that synthesizes the universal sulfate donor PAPS, as a critical regulator of SG lumen expansion. Our confocal and transmission electron microscopy (TEM) analyses of *papss* mutants reveal several defects in the apical membrane and the aECM in the SG, including defects in apical membrane expansion and microvilli formation, sparse aECM structures, and detachment of the aECM from the apical membrane. We also detect mislocalization of the key apical protein Crumbs and the recycling endosome marker Rab11 in *papss* mutants, suggesting defects in apical membrane organization and intracellular trafficking. The zona pellucida (ZP) proteins Dumpy (Dpy) and Piopio, as well as signals for wheat germ agglutinin (WGA; a lectin that recognizes mucin-type O-linked glycans), are disorganized in the SG lumen in *papss* mutants, suggesting a potential role for these proteins as key aECM components. Analysis of mutants for each of these components is ongoing, and the initial analysis for *dpy* mutants shows that disruption of *dpy* function results in irregular lumen diameter. In addition, we show that loss of *papss* also disrupts the basal ECM, allowing hemocytes to invade the SG epithelia. These findings shed light on the organization of the aECM and the mechanism of lumen expansion during tubular organ formation and add a new dimension to our understanding of aECM-mediated tube size control.

1125F **Homothorax is enriched in the adult Drosophila testis stem cell niche and is essential for its maintenance** Margaret de Cuevas, Erika Matunis Dept Cell Biology, Johns Hopkins Sch Med

In the adult Drosophila testis, germline stem cells (GSCs) and somatic cyst stem cells (CySCs) adhere to a small cluster of somatic niche cells called hub cells, which produce the signals that maintain and regulate adjacent stem cells. Hub cells normally divide only during embryogenesis and were thought to be quiescent and terminally differentiated in adult flies, but upon genetic ablation of all CySCs, they can be induced to divide, leave the hub, and convert into new, functional CySCs (Hétié et al., 2014). The adult Drosophila testis hub is thus a great genetic model for understanding niche plasticity and stem cell regeneration. Although some of the signals that regulate hub cells have been identified, much remains unknown about this small but essential cluster of cells. Using a single nucleus RNA-seq resource covering all of the adult Drosophila testis (Raz et al., 2023), we identified genes upregulated in hub cells and performed a small-scale RNAi-mediated knockdown screen, which revealed that *homothorax (hth)* is required to maintain the adult hub. *hth* encodes a homeodomain transcription factor that binds to another homeodomain transcription factor, encoded by *extradenticle (exd)*, and allows its nuclear import. Both are Hox cofactors that are essential for patterning of the Drosophila embryo but also have additional Hox-independent functions throughout development. We found that when *hth* is knocked down in adult hub cells, they convert to CySCs and are rapidly lost, followed as expected by loss of all GSCs and CySCs, which are not maintained in the absence of signals from hub cells. By contrast, when *exd* is knocked down in adult hub cells, some convert to CySCs but the hub remains intact, suggesting that *hth* has additional functions in the hub that are independent of *exd*. We are currently investigating potential downstream targets of *hth* and *exd* in the hub and asking whether or not they are required in other cell types within the testis niche.

1126F **Characterization of test-specific sugar transport and glycolysis genes in *Drosophila melanogaster*** Mark Hiller,

Bryanne Manley Goucher College

The genome of *Drosophila melanogaster* contains twenty-five genes that are annotated as SLC2 type sugar transporters, and five of the sugar transporters appear to be expressed only in the testis. During spermatogenesis, germline cells develop into sperm while completely surrounded by two somatic cyst cells. How sugars molecules transit the cyst cells and enter germline cells is not understood. Mutations in each of three predicted transporters, *sut3*, *sut4*, or *CG14605*, are fertile. One alternative model is that pyruvate generated in the cyst cells is converted to lactate which is then transported from cyst cells to the germline cells. The enzyme lactate dehydrogenase could convert lactate back to pyruvate which could be used to generate ATP. The *Drosophila* germline encodes one testis specific homolog of lactate dehydrogenase. Likewise, there are several genes that function in glycolysis that have testis specific homologs or testis specific spice forms. To assess the role of glycolysis and lactate transport during spermatogenesis, we are using RNAi in the germline and cyst cells of the testis to knockdown function of genes that potentially play a role in the lactate shuttle between cyst and germline cells.

1127F **The Regulation of Cell Fate Determinants by miR-190 During Asymmetric Cell Division in *Drosophila* Neuroblasts** Laura Galvan[1], Gerson Ascencio[2], Blake Riggs[3] [1]Biology Department, San Francisco State University, [2]Stanford University, [3]Department of Biology, San Francisco State University

Stem cells in the *Drosophila* brain, known as neuroblasts, generate most of the neurons in the brain by undergoing asymmetric cell division (ACD). ACD gives a product of two daughter cells with two separate identities facilitated by cell fate determinants such as Scribble, a scaffolding protein that regulates apical-basal polarity, Prospero, a transcription factor, and L(2)gl, a tumor suppressor protein. These determinants are partitioned asymmetrically to drive cell fate selection, with Prospero moving towards the basal end to promote cell differentiation in what will become the ganglion mother cell (GMC). The mechanism of ACD is highly conserved across all multicellular organisms. However, the organization and regulation of partitioning cell fate determinants is poorly understood. Recently, microRNAs (miRNAs) have been suggested to play a role in cellular functions such as cell fate specification and differentiation. Specifically, they modulate gene expression post-transcriptionally by inhibiting mRNA translation or inducing mRNA degradation, which comes from the complete or incomplete binding to the 3' untranslated region (3'-UTR) of specific mRNAs. Using the computational tool TargetScanFly we predicted a perfect conserved binding site in the 3'-UTR of Prospero to miR-190. Here we hypothesize that miR-190 regulates Prospero during ACD. Our qPCR data highlighted Prospero, a transcription factor, as a potential target transcript for miR-190. Our data shows an increased Prospero mRNA expression in miR-190 deficient embryos. This suggests that Prospero mRNA is regulated by miR-190 and induces more differentiated progenitor cells. Using immunohistochemistry and high-resolution confocal microscopy, we found that Pros is not localized correctly at the GMC in miR-190 mutant embryos. Instead, we discovered that Pros is localized around the two daughter cells during ACD. These findings suggest that miR-190 is a crucial regulator of Prospero by targeting Prospero mRNA and preventing Prospero from translating into a protein. Our results will help us understand how cell fate determination is established by a novel pathway and help close the knowledge gap regarding how cellular fate determination is organized correctly and how miRNAs can assist undifferentiated cells in obtaining their cellular determination.

1128F **The Mechanism of Histone Exchange between Lipid Droplets** Alicia Shipley[1], Asmita Dutta[2], Pakinee Phromsiri[2], Michael Welte[2] [1]Biology, University of Rochester, [2]University of Rochester

Lipid droplets (LDs) are highly conserved fat storage organelles with fundamental roles in lipid metabolism. They also sequester specific proteins, yet the underlying mechanisms are poorly understood. The best characterized example of such sequestration occurs in Drosophila embryos, where LDs handle certain histone proteins (H2A, H2B, and H2Av) in the cytoplasm. H2Av exchanges (transfers back and forth) between LDs via transient sequestration to the LD protein Jabba. This exchange mechanism paces the nuclear import of H2Av, which is critical for downstream regulation of chromatin assembly and gene expression. Exchange of H2Av is dynamic for the first 2+ hours of embryogenesis and then suddenly (in less than 15 min) stops and the transient Jabba-H2Av interactions become static. This switch in H2Av behavior occurs during the midblastula transition (MBT), a critical developmental time for the embryo. However, the mechanism driving the switch in H2Av dynamics and its biological function are unknown. Therefore, we aim to answer the central question: How is the exchange of H2Av between LDs temporally regulated? We are testing the roles of two candidate regulators of H2Av dynamics: Importin alpha 2 (Impα2) and Cyclin-dependent kinase 1 (Cdk1). Impα2 is necessary for H2Av exchange between LDs and is developmentally dephosphorylated just as H2Av exchange halts. Cdk1 activity has been proposed to mediate the effects of a molecular timer which modulates certain MBT events, including H2Av exchange. Because in mammals Cdk1 phosphorylates the ortholog of Impα2 at a site conserved in *Drosophila*, we hypothesize that Cdk1 controls Impα2's phosphorylation status which in turn affects H2Av dynamics. To test these ideas, we are generating phosphomimetic and phospho-null mutants of Impα2 and disrupt Cdk1 activity with inhibitors and cyclin knockdown. To follow Impα2's localization live, we have tagged it endogenously with GFP. As expected, we find that Impα2 is present on the nuclear envelope during interphase and enters the nucleoplasm during mitosis. In addition, during syncytial stages, Impα2 localizes to as-yet unidentified cytoplasmic puncta in a cell-cycle

dependent manner, dispersing during mitosis. Finally, when H2Av exchange stops in nuclear cycle 14, Impα2 becomes highly enriched on LDs as well as at the cellularization front. These dynamic spatial patterns suggest that Impα2 plays multiple roles in the early embryo.A long-term goal of this project is to determine which of these roles are relevant to H2Av exchange.

1129F **Hinge-Specific Cell Fate Plasticity and Ribosome Biogenesis in *Drosophila* After Radiation Damage** Caitlin Clark, Nathan Gomes, Tin Tin Su MCDB, CU Boulder

Ionizing radiation (IR) is used to treat approximately half of all cancer patients due to its ability to induce cell death. However, IR can also induce cancer stem cell-like properties in non-stem cancer cells, potentially prompting tumor regeneration and diminishing therapeutic success. Our published studies show we can model IR-induced stem cell-like properties in *Drosophila melanogaster* larval imaginal wing discs: IR-resistant cells from the hinge of the wing disc translocate and change their fate to replace the ablated pouch cells (Verghese and Su, 2016, PloS Biology, PMID: 27584613; Verghese and Su, 2018, PloS Genetics, PMID: 30462636). Genome-wide RNAseq analysis of disassociated wing discs cells identified several IR-induced gene expression changes specific to the hinge, such as up-regulation of genes that encode ribosome biogenesis factors. Functional testing showed that ribosome biogenesis is required specifically in the hinge for IR-induced cell fate change (Ledru et al, 2022, PloS Genetics, PMID: 34990447). We are currently expressing tagged ribosomes in the hinge so that we can use the tag to isolate ribosomes specifically from the hinge and perform Ribo-seq to identify mRNAs that become differentially ribosome-associated during a time course of regeneration. This identification may help us understand why increased ribosome biogenesis is required for IR-induced fate change.

1130F **METTL3 is required for Germline Function During *Drosophila* Spermatogenesis** Alannah Morse[1,1], Corinne Leighty[2], Emily MacLean[1], Hailey Kaba[2], Antonio L Rockwell[2] [1]Biology, Susquehanna University, [2]Susquehanna University

Work focused on RNA methylation which is a novel layer of gene expression regulation has contributed to the emerging field of epitranscriptomics. Epitranscriptomics is the study of how RNA modifications impact RNA metabolism, and ultimately cellular function. Our lab is focused on the methylation of N^6 residues on adenosine (m^6A), which is required for processes such as cell proliferation, differentiation and cell maintenance. Depletion of m^6A results in problems with numerous biological processes, for example neurogenesis, embryogenesis, and gametogenesis. Investigations focused on m^6A in gametogenesis have become prominent given the link between the modification and reproductive rates. Studies have shown errors in m^6A machinery result in gamete production issues in eukaryotes. Our lab is investigating the role of the enzyme that contains the catalytic domain of the m^6A complex, Drosophila Inducer of Meiosis 4 (*Dm* Ime4) also known as METTL3. Specifically, we are studying the role of METTL3 in spermatogenesis. Our work is currently focused on the later stages of spermatogenesis known as spermiogenesis, the development of spermatids into fully mature sperm. We find that germline knockdown of METTL3 results in spermatid bundle disassociation evident by phalloidin actin staining. This disassociation phenotype is coupled with a complete ablation of actin waste bags, a phenotype associated with late-stage progression through spermiogenesis. Additionally, we find germline knockdowns lack mature sperm in seminal vesicles. Data we collected from qPCR analysis suggests several genes required for normal spermiogenesis are downregulated in knockdowns. The spermatid and sperm phenotypes coupled with the downregulation of key spermiogenesis genes results in decreased fertility in our knockdowns relative to controls. Overall, our data suggests a critical role for METTL3 during germline development in *Drosophila*. Our findings may provide additional insight into the overall role of METTL3 and subsequently the m^6A modification in metazoan reproduction.

1131F **The stress response transcription factor Atf4 regulates wing development** Kenza Lahbabi, Deepika Vasudevan Cell Biology, University Of Pittsburgh

Emerging evidence shows that ‘stress response’ pathways are required for development and homeostatic function of various cell types. We recently discovered that hypomorphic mutants for Activating Transcription Factor 4 (Atf4), a stress response transcription factor, show reduced wing size and venation defects (PMID:34919148), leading us to determine Atf4 mechanisms in wing development. RNAi depletion of *Atf4* using Hh-GAL4 resulted in convex misshapen adult wings, indicating distorted growth in the wing disc’s posterior half in absence of *Atf4*. Depletion of *Atf4* in the dorsal compartment using Ap-GAL4 also resulted in misshapen adult wings. Contrastingly, depleting *Atf4* at the dorsal-ventral boundary using Wg-GAL4 showed no adult wing phenotypes, leading us to hypothesize that Atf4 is required for regulating the growth rate in the wing disc during development. Thus, a mismatch in growth of anterior/posterior or dorsal/ventral compartments due to *Atf4* loss leads to misshapen adult wings. Canonically, Atf4 expression is regulated by *Atf4* mRNA translation. We previously demonstrated the extra-ribosomal proteins, DENR and eIF2D, to be required for Atf4 translation (PMID:32938929). Thus, we also sought to determine if these factors were required for Atf4-mediated wing phenotypes. Our preliminary results indicate that depleting these translational factors in the posterior or dorsal compartments of the wing discs show misshapen wing phenotypes similar to Atf4. Together, our data suggest that Atf4 is required for ensuring proper rate of growth across wing disc compartments during development. We are currently investigating molecular mechanisms underlying such regulation, including whether loss

of *Atf4* impacts cell size, number, or differentiation.

1132F **The anilin Scraps, the kinesins Nebbish and Pavarotti, the citron kinase Sticky, and the Rho GTPase Tumbleweed are downstream effectors of Fox transcription factor-mediated cardiac progenitor cell divisions** Md Rezaul Hasan[1,2,3], Rajnandani Katariya[1,2,3], Kuncha Shashidhar[1,2], Mofazzal K. Sabbir[1,2], Andrew J. Kump[1,2,3], Manoj Panta[1,2], Kristopher R. Schwab[1,2,3], Mark H. Inlow[2,4], Shaad M. Ahmad[1,2,3] [1]Department of Biology, Indiana State University, [2]The Center for Genomic Advocacy, Indiana State University, [3]Rich and Robin Porter Cancer Research Center, Indiana State University, [4]Department of Mathematics and Computer Science, Indiana State University

Forkhead/Fox transcription factors (TFs) mediate multiple conserved cardiogenic processes in both mammals and *Drosophila*. We showed previously that the *Drosophila* Fox genes *jumeau* (*jumu*) and *Checkpoint suppressor homologue* (*CHES-1-like*) control three categories of cardiac progenitor cell divisions—asymmetric cell division, symmetric cell division, and cell division at an earlier stage—by regulating Polo kinase activity. Those observations raised two questions: whether other Fox TF-controlled genes mediating cardiac progenitor cell divisions are also regulated by both *CHES-1-like* and *jumu* in a *polo*-like manner and whether such Fox-regulated genes mediate all three categories of cardiac progenitor cell division or a subset thereof. By comparing transcriptional expression profiles of wild-type, *jumu* loss-of-function, and *CHES-1-like* loss-of-function mesodermal cells, we identified multiple genes transcriptionally activated by *jumu*, but not regulated by *CHES-1-like*. Phenotypic analysis of mutations of these exclusively *jumu*-regulated genes showed that two, the kinesin-encoding gene *nebbish* (*neb*) and the anilin-encoding gene *scraps* (*scra*), mediate only symmetric cardiac progenitor cell divisions and cell divisions at the earlier stage; while three other exclusively *jumu*-regulated targets, the citron kinase-encoding gene *sticky* (*sti*), the kinesin-encoding gene *pavarotti* (*pav*), and the Rho GTPase-encoding gene *tumbleweed* (*tum*), are required for all three categories of cardiac progenitor cell divisions. Synergistic genetic interactions between *scra*, *neb*, *jumu*, and *polo*; the absence of such synergistic interactions between either *scra* and *CHES-1-like* or *neb* and *CHES-1-like*; and the rescue of solely symmetric and earlier cardiac progenitor cell division defects in *jumu* mutants by ectopic cardiac mesoderm-specific expression of *neb* demonstrate that *scra* and *neb* comprise an exclusively *jumu*- and *polo*-regulated subnetwork mediating a specific subset of cardiac progenitor cell divisions. Using further pairwise genetic interaction assays, we are attempting to assess whether *sti*, *pav*, and *tum* are additional components of this subnetwork. Subsequently, by utilizing qPCR and rescue assays, we intend to determine where each of these subnetwork components is positioned topologically relative to one another. Collectively, our results illustrate how an individual regulator can utilize different combinations of downstream effectors to control distinct developmental processes.

1133F **Spargel/dPGC-1 is involved in chorion gene amplification and endoreplication** Mohammed Shah Jalal, Atanu Duttaroy Biology, Howard University

The vertebrate *PGC-1* (Peroxisome proliferator-activated receptor-gamma coactivator-1) family of transcriptional co-activators serve as the master regulators of energy metabolism and robust activators of mitochondrial biogenesis. Like in vertebrates, the *PGC-1* ortholog appears in Drosophila called Spargel/*dPGC-1* (*srl*), which is now known to be crucial for cellular growth, insulin signaling, and mitochondrial oxidative phosphorylation. Drosophila Spargel/dPGC-1 is abundantly expressed in adult ovaries because it is required in ovarian growth and female fertility. Here, we report that reduced maternal Spargel leads to defective dorsal appendages in the eggs and nuclear fallout in the syncytial embryonic stage, which suggests that maternally contributed Spargel plays a significant role in eggshell patterning and proper embryonic development. During oogenesis, Spargel is required for proper chorion gene amplification, where Cyclin E activity plays an essential role. Although there is a nuclear predominance of Spargel in the ovary, recent observations reveal that Spargel is dynamic as expressed in the cytoplasm of larval tissues. This cytoplasmic Spargel is active since ubiquitous and tissue-specific knockdown of *srl* causes growth arrest. Could this alternative subcellular localization of Spargel in somatic tissues be subject to the Post Translational Modification event? Such a possibility could be real since the global phosphoproteome profile of Drosophila embryos identified Spargel as a candidate for phosphorylation. Somatic clonal analysis shows complete loss of Spargel expression in growing somatic cells retards cellular growth, hence srl^{null} cell clones in salivary glands and fat body cells fail to attain the normal nuclear size. The absence of EdU incorporation in srl^{null} cell clones in the salivary gland indicates reduced endoreplication. Thus, our findings support that Spargel plays an essential role in eggshell biogenesis during oogenesis and endoreplication during larval development.

1134F **The deletion of *midline/H15* ventral leg enhancer causes pleiotropic abnormalities across tissues** Helen L Stott[1], Cody A Stevens[2], Julia Dodd[3], Alan Rozenblit[4], Nir Yakoby[2] [1]Center for Computational and Integrative Biology, Rutgers University - Camden, [2]Center for Computational and Integrative Biology, Rutgers University - Camden, [3]Forensic Science, Rutgers University - Camden, [4]Biology, Rutgers University - Camden

Animal development is controlled by a tightly regulated network of gene expression where genes are often reused across

multiple life stages and tissues. Our understanding of the mechanisms underlying the regulation of the same gene in multiple tissues is limited to only a few genes. Although we know that *cis*-regulatory modules (CRMs) provide spatiotemporal information for genes, we do not know how many are necessary for robust gene expression during development. Using the the *Drosophila* tandem paralogs *midline (mid)* and *H15, homologs of the* mammalian Tbx-20 gene, we examined the roles of three CRMs on *mid/H15* patterning and tissue development. Generally, *mid* and *H15* are expressed in overlapping domains. They sit within an isolated topologically associating domain (TAD) that contains no other genes. Using CRISPR-Cas9 gene editing, we deleted combinations of the three CRMs. Two (G04 and F11) were associated with *mid/H15* expression in the ovarian follicle cells, and one, the ventral leg enhancer (VLE) had been predicted to regulate their expression in the ventral domain of the leg disc. Interestingly, while the impact of G04 and F11 was only evident in the ovaries, the deletion of the VLE caused pleotropic effects on egg hatching, embryogenesis, leg and wing morphogenesis, larval survival, and eclosion. Specifically, wing abnormalities included spotting, issues with unfolding following ecolsion, and in rare cases extra appendage material projecting from the wing blade or margin. Abnormal legs phenotypes included the duplication of dorsal bristles and twisted or truncated tarsal segments. While the VLE deletion did not entirely eliminate *mid/H15* expression in the leg disc, reduction of their expression in the disc center can account for some of the leg phenotypes. We note, additional CRMs, yet to be found, should regulate *mid/H15* expression in this tissue. We will also discuss the effect of VLE deletion on gene patterning during other developmental stages.

1135F **Somatic gonadal precursors are transcriptionally related to the dorsal vessel** Megan B Butler[1], Ji Hoon Kim[1], Daniel Peng[2], Deborah J Andrew[1] [1]Cell Biology, Johns Hopkins University School of Medicine, [2]Biomedical Engineering, Johns Hopkins University

Primordial germ cells (PGCs) are specified during embryonic development and later differentiate into the mature cells of the adult testis or ovary. The cells that help the PGCs mature, and are essential for their survival, are the somatic gonadal precursors (SGPs). In *Drosophila melanogaster*, SGPs are thought to derive from the dorsolateral mesoderm of parasegments 10-12 during stage 11 of embryogenesis. Previous work suggests that SGPs come from a population of cells that also become the fat body (FB) – loss of *serpent*, a key FB transcription factor, leads to the production of more SGPs instead of FB. Multiple transcription factors contribute to the specification of SGPs within the mesoderm: *abd-A* expression is necessary to define a cellular environment permissive for SGPs, whereas *eya* and its activator *abd-B* are necessary to specify and maintain SGP fate. Loss of *tinman (tin),* an essential regulator for dorsal vessel (DV) development, decreases SGP number even though previous work reported that expression of *tin* in the SGPs was not detected. Thus, there is a potentially understudied relationship between cells that become the DV and those that become SGPs. Our lab generated single-cell RNA-sequencing data from early (stages 10-12) and late (stages 13-16) stages of embryonic organ formation. In the late-stage data, the SGPs clustered within the DV cells on the UMAP – not within the FB. We also saw mRNA expression of *tin* and other DV transcription factors in the SGP cluster. We confirmed expression and nuclear localization of Tin in the SGPs by immunostaining. Apontic, another DV marker, is also expressed in the SGPs as shown by scRNA-seq and immunostaining. While the presence of these DV fate-specifying transcription factors in SGPs does not give direct evidence that SGPs come from the DV, these results do suggest that there is a greater connection between the DV primordium and SGPs than previously appreciated. Using genetic manipulation on cell type-specifying transcription factors and quantitative cell tracking, we will investigate lineage relationships between the SGPs, DV, and FB. This study will advance our understanding of developmental mechanisms and how functionally differentiated organs - reproductive (SGP), circulatory (DV), and metabolic (FB) - are generated from common or adjacent primordia.

1136F **Phosducin-like Protein 3 is a critical regulator of spermatogenesis** Jennifer Mierisch[1], Christopher Petit[2], Claire C Chaikin[2], Grace C Flemming[2], Gabi C Rant[2], Anthony C Roukoz[2], Michaela C Marra[2], Elizabeth C Kojak[2], Stefan C Kanzok[2] [1]Biology, Loyola University Chicago, [2]Loyola University Chicago

Phosducin-like protein 3 (PhLP3) homologues have been shown to possess redox-activity and are hypothesized to function as a co-chaperone in the folding of cytoskeletal proteins. However, the relationship between these two functions is unclear. The *Drosophila melanogaster* homolog of PhLP3 is encoded by the previously uncharacterized *CG4511* gene and will be referred to as Phlp3 here. We find that Phlp3 plays a role in the regulation of spermiogenesis in *Drosophila melanogaster.* Males homozygous for a P-element insertion in the 5' UTR of *Phlp3* exhibit decreased *Phlp3* expression, infertility, and a failure to produce mature sperm. Further examination of these *Phlp3*$^{-/-}$ testes reveals that actin-based individualization cones are absent, as are the needle-like nuclei indicative of mature sperm. Spermatid nuclei fail to align, appearing scattered throughout the syncytium. Microtubule-rich dense complexes (DCs), which serve as a scaffold for nuclear shaping, appear reduced in size, suggesting a reduction in the number of microtubules in the DC. Thus, nuclei may fail to fully elongate due to a defective DC. As a result, mature sperm are not produced in *Phlp3* mutants and seminal vesicle size is severely reduced. Excision of the P-element restores male fertility, spermiogenesis, and seminal vesicle size. Given our observations and the hypothesized role of PhLP3 proteins as co-chaperones for cytoskeletal proteins, we hypothesize

that Phlp3 functions to regulate the microtubule dynamics that are vital for DC formation and the nuclear-shaping process during spermiogenesis, as well as the function of the actin-based individualization complexes. We are currently examining the importance of thioredoxin activity for Phlp3 function in spermatogenesis, as well as the effects Phlp3 mutation on the cytoskeleton using Transmission Electron Microscopy. PhLP3 proteins are well-conserved in humans, suggesting that our results on Phlp3 function in flies. Therefore, we are testing the ability of the human homologue of Phlp3, Txndc9, to functionally substitute for Drosophila Phlp3 during spermatogenesis. This

1137F **Determining how Doublesex and sex-specific steroid hormone signaling control gonad development** Samantha C Goetting[1], José Pac Cordero[2], Mark Van Doren[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

In the animal kingdom, most species exhibit sexual dimorphism, or phenotypic differences between the sexes. In *Drosophila*, the key factor controlling sexual dimorphism is Doublesex (Dsx). Dsx is the founding member of the Doublesex/Mab-3 Related Transcription Factor (DMRT) family and is conserved in many animals, including humans. Our lab has shown that Dsx regulates sex-specific steroid hormone Ecdysone (E) signaling through female-specific Ecdysone Receptor (EcR) expression, and that this functions to promote ovary development and repress testis development. Bioinformatic analyses have been used to predict Dsx targets, and many are transcription factors that contain a BTB (Broad-complex, Tramtrack, and Bric-a-brac) domain that promotes homo- and heteromeric BTB protein interactions. An RNAi screen of BTB domain transcription factors revealed that several are important for normal gonad development. Here we focus on three of these factors: *broad (br), chinmo (chronically inappropriate morphogenesis)* and *mamo (maternal gene required for meiosis)*.

Knockdown of *br* in the somatic gonad causes severe somatic cell defects in the ovary from third larval instar (L3) onwards. Loss of *br* or *mamo* leads to severely disorganized adult ovaries and loss of egg production, with a female-specific phenotype. Conversely, loss of *chinmo* leads to formation of follicle-like cells in the developing testis. Upon examining Mamo and Chinmo expression during development, I found that Mamo is female-specific while Chinmo is male specific. Previous studies revealed a relationship between *br, chinmo,* and *mamo*. In the wing disc, a mid-L3 Ecdysone pulse mediates a Chinmo-to-Br switch; in neurons, temporal expression of Chinmo or Mamo can specify neuronal fates. These observations provide useful groundwork for their potential roles in the gonad. Given these data, I hypothesize that *br* and *mamo* are important for proper ovary development and function, and that they lie downstream of Dsx and/or E signaling to help maintain a female sexual fate. I plan to characterize what cell types are affected in the absence of *br, chinmo,* and *mamo* using cell-specific markers and examine how altering *dsx* and E signaling affects expression of these genes. This work will expand current knowledge on how DMRTs control sexual dimorphism and could uncover genes critical for sexual identity in both flies and mammals. Due to the universal nature of DMRTs in controlling sex-specific development, understanding their targets and how they function is of great importance for reproductive health and has the potential to broaden our knowledge of human infertility.

1138F **Peroxisome Proliferator Activated Receptor Gamma Co-activator-1 (PGC-1): Functional convergence and divergence between vertebrates and invertebrates** Swagota D Roy[1], Mohammed Shah Jalal[2], Sabarish Nagarajan[3], Atanu Duttaroy[2] [1]Biology, The Howard University, [2]Biology, Howard University, [3]Genetics/ Biotechnology, University of Wisconsin, Madison

Peroxisome Proliferator Activated Receptor Gamma Co-activator-1 (PGC-1) is a canonical transcription co-activator in all Chordates and misregulation of these coactivators leads to a wide range of diseases, including cancer and lethality. In vertebrates, the *PGC-1* (Peroxisome proliferator-activated receptor-gamma coactivator-1) family of transcriptional co-activators consists of *PGC-1α*, *PGC-1β*, and *PRC* (PGC-1-related Coactivator) are well known since they serve as master regulators of energy metabolism and a robust activator of mitochondrial biogenesis. In addition to bioenergetics, the PGC-1 group of proteins is also involved in myriads of activities in specific tissues like gluconeogenesis and anti-apoptosis in the liver, thermogenesis in adipose tissues, fiber-type switch in skeletal muscles, etc. Many invertebrates including *Drosophila* carry a single homolog of PGC-1 named Spargel/dPGC-1 that shows many functional convergence and divergences with vertebrate PGC-1. Since Drosophila homolog is prevalent as a single copy gene could it circumvent the redundancy issue in vertebrates? Indeed, significant functional similarities are shared between vertebrate and invertebrate PGC-1's based on their role in mitochondrial functions and biogenesis, gluconeogenesis, growth, and cancer. Development is delayed in both Spargel and PGC-1 related coactivator (PRC), a member of PGC-1 gene family and complete knockouts of *srl* and *PRC-1* are embryonic lethal. In terms of divergence, we not only established that Spargel plays an essential in *Drosophila* oogenesis but the critical role of Spargel RRM and RS domains are established in oogenesis. Spargel is a maternal effect gene, which is potentially involved in Endoreplication in polytene cells. These specific aspects of PGC-1 biology are to be explored in vertebrates. Therefore, a complete functional comparison between Spargel/dPGC-1 and PGC-1 will pave the way to uncover unexplored functional equivalence in mammals.

1139F **Regulation of growth and patterning in the developing eye of *Drosophila melanogaster* by the Hippo pathway**

coactivator *yorkie* and dorsal selector *defective proventriculus* Rohith BN[1], Amit Singh[2,3,4], Madhuri Kango-Singh[1,3,4] [1]Growth Regulation and Signalling Laboratory, Department of Biology, University of Dayton, [2]Drosophila Development and Disease Laboratory, Department of Biology, University of Dayton, [3]Premedical Program, University of Dayton, [4]Integrative Science and Engineering Center, University of Dayton

The developing eye of *Drosophila* is a well-established model for studying developmental genetic processes and growth regulation. The developmental genetic networks discovered in *Drosophila* are highly conserved in all animals including higher mammals. Our long-term goal is to understand the molecular basis of Dorsal-Ventral patterning and growth in the eye by interactions of the dorsal selector genes and growth regulatory genes. We recently identified *defective proventriculus* (*dve*) as a candidate for dorsal-ventral eye patterning that acts as a transcriptional regulator. Gain of function of Dve, results in eye suppression, while loss of function of *dve*, exhibits dramatic eye enlargement phenotypes which raised an interesting question, whether the dorsal patterning gene *dve*, apart from its main function of specifying cells fate, plays a dual role in regulating growth during eye development in *Drosophila*? We hypothesized that Dve may interact with the Hippo growth regulatory pathway to control patterning and growth of the eye. We tested the interactions between Hippo pathway and dorsal-ventral patterning using the GAL4-UAS system and MARCM. To investigate whether *yki* is involved in *dve* domain growth, we used GAL4 drivers in the eye like Dve Gal4 to drive the spatiotemporal expression of transgenes in the dorsal eye, and Ey-Gal4 and GMR-Gal4 to test the effects of Dve and Yki modulations before and after MF formation in the larval eye antennal disc. Initial data suggests genetic interactions between Hippo and DV patterning genes are important in early eye development. We have tested *wingless* (*wg*) a known and conserved Hippo downstream target for Dve and Yki mediated effects using reporter assays, clonal analysis and qRT-PCR- based approaches; and our results will be discussed.

1140F **Transcriptional co-repressor Atrophin regulates Hippo pathway target genes in *Drosophila*** Deimante Mikalauskaite[1], Cordelia Rauskolb[2], Tom Lehan[2], Srividya Venkatramanan[2], Kenneth D Irvine[2] [1]Waksman Institute, Rutgers University, [2]Department of Molecular Biology and Biochemistry, Waksman Institute, Rutgers University

The Hippo signaling pathway controls expression of target genes through its downstream effector, the transcriptional co-activator Yorkie (Yki). With our expanding knowledge of Hippo pathway activity, other components regulating expression of Yki target genes still need to be identified. Previous studies suggest that the transcriptional co-repressor Atrophin could regulate expression of the Yki target gene *four-jointed*.

Using gene knock down and overexpression approaches we investigated whether Atrophin contributes to Hippo signaling in the wing imaginal disc, and what molecular mechanisms underlie this regulation. We found that Atrophin regulates multiple Hippo pathway target genes. Interestingly, our results show that Atrophin represses the expression of Yki target genes in the distal wing and activates them in the proximal wing. When we investigated Atrophin's effect on Yki, we found that Atrophin knock down resulted in decreased Yki nuclear levels throughout the wing, while overexpression had an opposite effect, suggesting that Atrophin controls Yki nuclear localization. This result implies that Atrophin regulates Hippo pathway activity. We further identified that depletion of Atrophin leads to changes in component levels of Fat-Dachsous (Ft-Ds) signaling, which controls Hippo pathway activity.

We are now investigating how Atrophin regulates the levels of the Ft-Ds factors. In addition, we plan to identify direct transcriptional targets of Atrophin. These studies will help us understand how Atrophin exerts its regulatory function on the components of the Ft-Ds network and explain its downstream effects on Yki target gene expression.

1141F **Functional analysis of a Drosophila Hox gene enhancer essential for segment-specific sense organ development** Xinyuan Liu, Teresa V Orenic Biological Sciences, University of Illinois at Chicago

Hox genes encode conserved transcription factors (TFs) that specify segmental or regional identity along the anterior-posterior (A/P) axis of developing animal embryos and also function during later developmental stages in patterning of limbs and other organs. During embryonic development, the Hox TFs function at the top of the hierarchy that controls intra-segmental patterning to generate differences in segmental/regional patterning. On the other hand, recent studies in Drosophila and other insects suggest that the Hox genes are targets of intra- segmental patterning genes and function downstream of these genes to generate morphological differences among limbs. We are investigating the regulation of the Hox gene *Sex combs reduced (Scr)*, in response to the intra-segmental patterning networks that control development of the Drosophila adult legs. *Scr* is expressed throughout legs of the first thoracic segment (T1) legs, but its expression is elevated in defined domains of developing legs within the primordia of a group sense organs, the transverse bristle rows (TBRs). We have identified an *Scr* enhancer (*ScrE*) that drives expression in the TBR primordia and is required for TBR development in T1 legs. Furthermore, the proximal/distal (P/D) patterning genes *Distalless* (*Dll*), *dachshund* (*dac*) and *bric-a-brac1/2* (*bab1/2*) regulate *Scr* expression through the enhancer. Dll, a homeodomain (HD) TF, activates *Scr* expression through multiple sites dispersed throughout the *ScrE* enhancer. Dac

is a conserved TF that is required for upregulated expression of both *Scr* and *ScrE-GFP.* The *bab* locus contains 2 paralogous genes, *bab1* and *bab2.* Previous studies suggested Bab1 function is not essential for leg development. However, we recently found that both Bab1 and Bab2 are essential for TBR patterning and *Scr* distal repression in T1 legs. In addition, *ScrE* is responsive to repression by Bab1/2, and potential Bab-response sequences have been mapped to a 78bp conserved block within *ScrE*. An in vivo functional analysis of these sequences is in progress to determine the necessity of these sites for *Scr*expression and patterning of T1 leg sensory organs. This investigation will provide insight into Hox gene regulation of segment-specific sense organ patterning in response to leg P/D patterning TFs.

1142F **Characterization of novel *Drosophila* Egf receptor signaling targets with roles in eggshell morphogenesis** Kayla Eckrote, Autumn Bullek, Sara Delgado, Lisa Kadlec Biology and Earth Systems Science, Wilkes University

Drosophila epidermal growth factor receptor (Egfr) signaling plays a critical role in many aspects of development including oogenesis, embryogenesis, and proper development of wing and eye tissues. For example, during wing development Egfr signaling helps specify vein tissues, and in the ovary Egfr signaling is known to establish the body axes during oogenesis. Microarray screens by our lab and others have identified potential downstream transcriptional targets of the Egf receptor using the *Drosophila* ovary as a model system. Our initial work compared gene expression in fly ovaries where the activity of the Egfr pathway was reduced (gurken mutant), wild-type (OreR), or constitutively active (CY2/λTop). We have employed a number of approaches to further investigate the expression, biological function, and mechanism of action of a subset of putative genes of interest, focusing primarily on genes of previously unknown function. A small-scale functional screen using available collections of UAS-RNAi transgenic flies and P-element insertion lines was used to investigate the possible functions of a group of these novel EGFR-responsive genes. This screen has identified multiple genes activated by EGFR signaling that appear to have roles in the morphogenesis, rather than the patterning, of eggshell features. Gene mutant/knockdown phenotypes include severely decreased chorionic integrity, shortened eggs, and various dorsal appendage malformations, as well as decreased fertility. We have also used the CRISPR-Cas9 system to create mutations in some of these genes, with a goal of generating null mutant lines. Characterization of these mutants has so far revealed expected (previously observed) phenotypes, and in some cases additional eggshell phenotypes not seen with the original knockdown or P-element flies. We are continuing to characterize our most recently generated CRISPR lines, as well as using existing CRISPR mutants for further study and characterization of our genes of interest.

1143F **Transcriptional pausing mechanism: A common link for two independent homeostatic pathways of apoptosis and autophagy** Anuradha V Chimata[1], Hannah Darnell[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

During development, cell death mechanisms like apoptosis and autophagy work independently in regulating cellular homeostasis. Cellular homeostasis, crucial for organ growth and development, is regulated by signals generated by activation of specific gene cascades. These signals are governed at a transcriptional level by several mechanisms including transcriptional pausing. Motif 1 binding protein (M1BP), a functional homolog of human ZKSCAN3, is a transcription pausing factor that binds to several developmentally regulated genes. Recently, we have shown that loss-of-function of M1BP results in a “reduced-eye” or “no-eye” phenotype due to cell death. We employed a genome-wide forward genetic screen strategy to identify downstream targets of M1BP, which resulted in identification of members of evolutionarily conserved Jun-amino-terminal-(NH2)-Kinase (JNK) signaling pathway as modifiers of the “reduced-eye” or “no-eye” phenotype of $MIBP^{RNAi}$. JNK pathway is a pro-death pathway that is a master regulator of different types of cell death. We found that downregulation of M1BP resulted in activation of JNK signaling pathway, which resulted in induction of caspase-dependent cell death. However, blocking caspase-dependent cell death did not provide a complete rescue of “reduced-eye” phenotype of M1BP downregulation. Previous studies in mouse models have shown that double knock-out mice for apoptosis genes still showed delayed but increased cell death via compensatory autophagy mechanism. Therefore, we looked for other cell death mechanisms and found that autophagy is also upregulated when M1BP is downregulated. Interestingly, JNK is also known to induce autophagy, a caspase-independent cell death mechanism. Blocking both apoptosis and autophagy along with M1BP downregulation resulted in a significant rescue of M1BP “reduced-eye” phenotype. Our study revealed for the first time that M1BP activates both apoptosis as well as autophagy in JNK dependent manner. Here, we demonstrate a novel cellular homeostasis mechanism where transcriptional pausing is regulating these two genetically distinct modes of cell death during eye development to promote cell survival and regulate cellular homeostasis.

1144F **Unraveling adherens junction signaling complexity in the oral epithelia during palatogenesis** Juliet S King[1], Kendall Lough[2], Akankshya Jena[2], Scott Williams[3] [1]Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, [2]University of North Carolina at Chapel Hill, [3]Pathology and Laboratory Medicine Department, University of North Carolina

at Chapel Hill

Cleft palate (CP) is among the leading congenital diseases in the US and across the globe. CP results from a failure to separate the oral and nasal cavities during embryogenesis, a process known as palatogenesis. One contributing factor to the widespread nature of CP is the over 200 genes in humans and mice that when mutated disrupt palatogenesis. Among the CP-linked genes, components of the adherens junction (AJ) complex expressed in the oral epithelia, have garnered recent attention. Mutations in genes that encode AJ receptors, like cadherins (*CDH1*) and nectins (*NECTIN1*), are associated with CP in humans. However, we still do not understand the mechanism by which AJ receptors facilitate palate closure. To address this, our lab has disrupted nectins and cadherins, as well as their downstream signaling effectors, to determine their effect on palate closure. Currently, we are focused on segregating the relative contribution of AJ signaling across the two oral epithelial layers, the progenitor basal keratinocytes and the protective periderm layer. To do so, we are knocking down and knocking out AJ proteins in both a periderm-specific manner as well as a whole oral epithelial loss. Our lab utilizes two genetic manipulation techniques: 1) traditional Cre/LoxP mediated gene knockout as well as 2) *in-utero* guided lentiviral injections that transduce the outer ectoderm with short-hairpin RNAs (shRNAs) and gene expression constructs. Preliminary evidence has suggested that periderm-specific loss of cadherin 1, aka E-cadherin (*Cdh1*) demonstrates a similar palatal fusion defect as *Cdh1* knockdown in both oral epithelial layers. This suggests that E-cadherin signaling is at least in part required in the periderm. Future directions are focused on determining if periderm-specific loss of afadin (*Afdn*), the downstream nectin binding partner, can drive CP at the same penetrance as whole epithelial *Afdn* loss does. These experiments aim to discern between two possible functions of the periderm: A passive function, where the periderm simply protects underlying basal keratinocytes from unwanted intraoral adhesions. Or an active role, where the periderm participates in palatal fusion through AJ-based cell adhesion programs. Moreover, this work continues to elucidate important signaling mechanisms that regulate palatogenesis with the potential to generate novel models of CP in mice.

1145F **Understanding the aberrations in male reproduction caused by the mouse t-haplotype.** Ana M Velasquez[1,2], Camilo Hernandez-Aviles[3], Luisa Ramirez-Agamez[3], David W Threadgill[1,2] [1]Interdisciplinary Graduate Program in Genetics and Genomics, Texas A&M University, [2]School of Medicine, Department of Cell Biology and Genetics, Texas A&M University, [3]School of Veterinary Medicine and Biomedical Sciences, Texas A&M University

The mouse t-complex is one of the most well-known examples of naturally existing super-Mendelian inheritance. Despite decades of intense research, the mechanism underlying such special inheritance pattern is yet to be fully understood. The t-haplotype comprises a series of four non-overlapping inversions on mouse Chromosome 17. It has been established that the t-haplotype accomplishes biased transmission by disrupting motility of wild type sperm in heterozygous males.

Many genes that play important roles in such dysregulation have been mapped and identified. Nonetheless, it is yet to be established exactly how these gene products interact together to produce the phenotypes observed. Several studies have detected a series of morphological and physiological abnormalities present in the sperm of t-carrying males. However, some of these studies contradict each other.

A likely explanation for the differing results is the use of different t-carrying mouse strains. There are numerous versions of either partial or complete t-haplotypes that vary in inheritance rates ranging from 20% to 99% between t-haplotypes. Additionally, some of these strains carry a homozygous lethal gene that makes sperm analyses challenging, while for the remaining strains homozygous males are sterile.

This study aims to better understand the reproductive aberrations on males carrying the tw2 haplotype. The tw2 strain was originally captured in 1946 and has been maintained in laboratory since. This specific haplotype is advantageous as these mice carry a complete t-haplotype with 95-99% inheritance rates but lack lethal mutations allowing for the study of sperm from homozygous males. In addition, this specific haplotype has recently attained attention from scientists as a potential tool for genetic biocontrol of invasive rodents. In collaboration with the Texas A&M University Theriogenology Laboratory, we are studying a series of morphological, physiological, and molecular parameters in reproductive tissues of tw2 mice to identify the underlying issues resulting in homozygous male sterility, as well as aberrations in heterozygous males.

Results from this study will guide scientists on their use of the t-haplotype, and specifically the tw2 haplotype, for mouse eradication purposes, as well as inform future experiments to better understand of the mouse t-haplotype.

1146F **Temporality and Mechanisms of Retinogenesis in Human Retinal Organoids** Benvindo Chicha, Robert Johnston Biology, Johns Hopkins University

Human vision is dependent on the retina, a multilayered tissue that detects, processes, and relays light information. Vision

begins with the detection of light by photoreceptors. This light-induced signal is transmitted via interneurons including bipolar cells, amacrine cells, and horizontal cells, to retinal ganglion cells. All light information converges at retinal ganglion cells before being transmitted to the brain. In addition to these neurons, Müller glia span the retina and are responsible for architectural and metabolic functions. The numerous neuronal types of the retina are generated at different overlapping timepoints, yet a high resolution timeline of cell birth, maturation, and death in the developing human retina has not been achieved, due to the experimental inaccessibility of human fetal retinal tissue. Moreover, the spatiotemporal mechanisms controlling neuronal development in the human retina are poorly understood. It is unclear if the temporality of cell fate specification reflects the birth timing or maturation rates of these cells. The main goal of this study is to identify temporal mechanisms governing the generation and maturation of neurons in the human retina using human retinal organoids.

To assess the timing of neuronal type birth, maturation, and death, I am using nucleotide analogs (i.e. EdU) to label newly replicated DNA for 48 hours every 7-days across organoid development. Whereas dividing progenitor cells will dilute the label over time, post-mitotic cells will retain this label, enabling me to track cells born at distinct times. While larger windows are preferred, my preliminary experiments showed that EdU is toxic when administered in >48-hour pulses, limiting the length of the temporal windows. In a parallel approach, I am developing a transgene-based strategy to birth date cells with H2B-GFP in wider windows. Mitotic cells will divide and dilute the label while neurons born in the window will retain the H2B-GFP. this system, allows more fine-tuned control and adaptability for later experiments.

The Johnston lab found that retinoic acid (RA) acts early and thyroid hormone (TH) acts late during retinogenesis to specify photoreceptor fates. To test how these signals affect the timing of cell generation, I am examining changes in cell birth dates upon changes in signaling environments. These studies will determine how signaling pathways govern the timing of cell type generation and cell fate specification decisions.

1147F **Evidence for essentiality of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of phytohormone ethylene in Arabidopsis thaliana** Husan Turdiev[1], Bret Cooper[2], Caren Chang[1] [1]Cell Biology and Molecular Genetics, University of Maryland, [2]Soybean Genomics and Improvement Laboratory, USDA ARS

Ethylene is a gaseous phytohormone that has major effects on plant growth and development, including seed germination, root growth, fruit ripening, and senescence, as well as tolerance to biotic and abiotic stresses. In angiosperms, ethylene is produced in a two-step reaction: S-adenosylmethionine (SAM) is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase (ACS), and ACC is then converted to ethylene by the enzyme ACC oxidase (ACO). Recent evidence suggests that ACC itself can induce responses independent of its role as the ethylene precursor. However, the roles of ACC as a potential signal are unclear, in part because there is no *acs* null mutant. In *Arabidopsis thaliana*, the only available *acs* octuple mutant shows incomplete knock-down of two of the eight Arabidopsis *ACS* genes by artificial micro-RNA silencing (Tsuchisaka et al., *Genetics* 2009), and we have found that this “octuple” mutant still produces ACC. We therefore aimed to create a true *acs* null mutant in Arabidopsis by utilizing a multiplex CRISPR-Cas9 system (Stuttmann et al., *Plant J.* 2021) to target all eight *ACS* genes. Surprisingly, we have only been able to generate an *acs* septuple mutant lacking all but a single *ACS* gene. We found that seeds of the *acs* septuple mutant consistently fail to germinate when they carry the CRISPR-Cas9 transgene, suggesting that editing of all eight *ACS* genes is responsible for the failure to germinate. This contrasts with the apparent dispensable role of ethylene production (Li et al., *Mol. Plant* 2022) and raises the possibility that ACS function is essential for plant growth. Currently, we are testing whether ACC can rescue the seed germination defect and are designing a conditionally viable *acs* octuple mutant to investigate the requirement for *ACS* activity. Our work will potentially reveal a novel ACC function in plants.

1148F **Wnt4 and ephrinB2 instruct apical constriction via Dishevelled and non-canonical Wnt signaling** Jaeho Yoon, Ira Daar NCI

Apical constriction is a cell shape change critical to vertebrate neural tube closure, and the contractile force required for this process is generated by actin-myosin networks. The signaling cue that instructs this process has remained elusive. Here, we identify Wnt4 and the ephrinB2 transmembrane ligand as playing instructive roles in neural tube closure as members of a signaling complex we termed WERDS (Wnt4, EphrinB2, Ror2, Dishevelled (Dsh2), and Shroom3). Disruption of function or interaction among members of the WERDS complex results in defects of apical constriction and neural tube closure. The mechanism of action involves Wnt4 binding the non-canonical Wnt receptor, Ror2, and enhancing an interaction with the transmembrane protein, ephrinB2, through the main Wnt scaffold protein, Dsh2. This complex in turn recruits the adaptor protein Shroom3, which activates Rho-associated kinase (Rock) to induce apical constriction. Remarkably, we also discovered that a key part of this mechanism is that ephrinB2 antagonizes Wnt/β-catenin signaling through the interaction with Dsh2, which switches Dsh2 function from canonical to non-canonical Wnt signaling that is required for proper neural tube closure.

1149F **Meiosis II Spindle disassembly requires two distinct pathways** Xheni Mucelli, Brian C. Seitz, Maira Majano, Zoey

Wallis, Ashley C. Dodge, Catherine Carmona, Matthew Durant, Sharra Maynard, Linda S. Huang Biology, University of Massachusetts Boston

Budding yeast undergo sporulation when starved of adequate nutrients. Sporulation involves meiosis and the packaging of the haploid nuclei into spores. At the end of meiosis II, *Saccharomyces cerevisiae* cells must disassemble the meiosis II spindle and undergo cytokinesis; these two events must be coordinated. Meiosis II cytokinesis occurs by prospore membrane closure: the prospore membrane is a newly synthesized membrane that grows around each of the haploid nuclei for cellularization of the developing spore within the mother cell. *SPS1*, which encodes a STE20-family GCKIII kinase that acts downstream of the Hippo-like kinase encoded by *CDC15*, and *AMA1*, which encodes a meiosis specific activator of the Anaphase Promoting Complex, are required for both spindle disassembly and cytokinesis in meiosis II. To better understand the regulation of spindle disassembly and cytokinesis in meiosis II, we examined roles of *SPS1* and *AMA1* in exit from meiosis II. We find that the prospore membrane closure defects of both *sps1Δ* and *ama1Δ* cells do not result from the failure of spindle disassembly. Instead, these two processes are regulated independently of one another by *SPS1* and *AMA1*. We also find that *sps1Δ* and *ama1Δ* cells exhibit distinct defects in spindle disassembly. By examining known microtubule-associated proteins important for spindle disassembly in mitosis, we find *AMA1*is required for the removal of Ase1 and Cin8 from the meiosis II spindle, while *SPS1* is required for proper removal of Bim1. Our data indicate that the two pathways represented by *SPS1* and *AMA1* are required for different aspects of meiosis II spindle disassembly. Furthermore, these microtubule associated proteins are regulated by meiosis-specific regulatory proteins that act in similar pathways as the pathways used to regulate mitotic spindle disassembly.

1150F **Control of Yeast Cell Fusion by Regulated Secretion** Ursula Machi[1], Emily Mazur[1], Annika Sundlof[2], Jean Smith[3], Mark Rose[2] [1]Georgetown University, [2]Georgetown University - Washington, DC, [3]Stetson University

Cell-cell fusion is a critical event in many systems, including muscle development, fertilization, and immune response. In yeast mating, partner cells must first degrade the intervening cell wall material before plasma membrane fusion can be achieved. Cell wall removal is carried out by secreted remodeling enzymes including Scw4, Gas1 and Scw10, which have been implicated in mating previously or in this study. The action of these enzymes must be tightly controlled, as premature or ectopic cell wall degradation would cause cell lysis. Yeast cell fusion requires continuous secretion, as revealed by mutations in the standard secretory pathway. Electron microscopy demonstrated the appearance of mating-specific vesicles in pheromone-responding cells and at the Zone of Cell Fusion (ZCF) in prezygotes. The vesicles dissipate after fusion is complete. The behavior of the vesicles appeared to be regulated by proteins required for fusion including Fus2, Rvs161, and Cdc42. Deletion of *FUS2* and function specific mutations in *RVS161* and *CDC42* appear to block vesicle fusion with the plasma membrane (as judged by their retention at the ZCF) and cause defects in subsequent cell wall degradation, but do not show evidence of defects in general secretion. Interaction between these proteins is required for cell fusion, likely through facilitation of secretion of these vesicles. To study the possibility of a mating-specific regulated secretion pathway in yeast, we developed a single-cell microscopic secretion assay using a Fluorogen-Activated Protein (FAP) tag fused to Scw4, Scw10 and Gas1. Secretion is assayed using a membrane-impermeant fluorogenic dye; binding to secreted FAP generates a fluorescent signal. Secretion of FAP-Scw4 and FAP-Gas1 at the ZCF is dependent on Fus2, whereas FAP-Scw10 secretion is not, revealing both general secretion and Fus2-regulated secretion at the ZCF. Blocking the interaction between Fus2 and Cdc42 with function specific alleles altered the secretion pattern of FAP-Scw4 and FAP-Gas1. Whereas WT cells largely had a singular focused burst of secretion at the ZCF, loss of the interaction caused multiple transient secretion foci. As Scw4 and Gas1 expression is high in mitotic cell and shut off during mating, we conclude that Scw4 and Gas1 are likely preloaded into the mating-specific vesicles, which are specifically secreted during conjugation to help effect cell wall removal. Rather than being required for secretion per se, Cdc42 facilitates Fus2 localization to ensure wall degradation cargo is secreted in a concentrated fashion, capable of overcoming cell wall repair regulated by the Cell Wall Integrity Pathway.

1151F **Zebrafish as a new model to analyze tooth development and homeostasis** Kanako Inoue[1], Kiyohito Taimatsu[2], Brant Weinstein[2] [1]NIH, [2]NICHD, NIH

Although the process of tooth development is highly conserved from fish to mammals, the process of tooth development has not yet been fully elucidated because it begins during embryonic stages and teeth are opaque hard tissues that are relatively inaccessible to observation and experimental. Until now, tooth analysis methods have been conducted only on isolated teeth, such as histological sectioning and *ex vivo* culture of tooth germs, and the behavior of cells involved in the development and the three-dimensional (3D) localization of surrounding tissues such as blood vessels have not been elucidated to any significant degree. We are developing the zebrafish as a powerful new model organism for *in vivo* study of tooth development.

Zebrafish have pharyngeal teeth attached to the fifth branchial arch in the pharynx. Zebrafish dentition consists of three rows of teeth on each side for a total of 11 teeth - five in the ventral row, four in the mediodorsal row, and two in the dorsal row. Zebrafish pharyngeal teeth are constantly replaced every about two weeks throughout life, allowing observation of multiple

stages of tooth development at all times in the same animal. Our group has pioneered a new imaging technique using a novel clearing reagent to render the teeth and bones of the zebrafish optically clear. This allows analysis of tooth 3D structure and the dynamics of cell populations involved in tooth development. We have performed high-resolution imaging of fluorescent reporter lines labeling a variety of tooth cell types including odontoblasts, dental epithelial cells, and surrounding mucous cells, revealing their detailed 3D distribution in the dental pulp and surrounding structures. We have succeeded in 3D imaging the differentiation of dental epithelial cells into enamel-secreting ameloblasts and have even visualized minute microstructures such as dentin tubules in odontoblasts. Together, our work reveals the comprehensive three-dimensional structure of the developing and adult tooth, from the pulp to the surrounding tissues, underscoring the utility of the zebrafish as a powerful model for analyzing tooth development and homeostasis.

1152F **Vgll3 as a Transcriptional Regulator in Embryonic Hindbrain Cell Fate Pathways** Cameron E Bennett, Yong-Il Kim, Rebecca O›Rourke, Charles Sagerström Pediatrics-Developmental Biology, University of Colorado Anschutz Medical Campus

Understanding what drives neural progenitor cell fate decisions is a key question in developmental biology as incorrectly timed and placed neural circuitry is associated with neurodevelopmental disorders. One proposed mechanism for controlling spatiotemporal neural development are neuromeres, called rhombomeres in the hindbrain. These seven or eight segmented regions are highly conserved across vertebrates and have restricted cell lineages, but how they form remains unknown. Previous investigation identified pre-rhombomeres, however, it was uncertain if they were an artifact of mechanical cell migration forces or represented progenitors with distinct fates. Previous work in the field showed that mutations in transcription factor Mafba (Mafb in Humans) inhibit the formation of distinct r5 and r6 rhombomeres, resulting in the retention of rX, a distinct progenitor domain, in their place. This finding is some of the only molecular evidence in the existing body of literature for the existence of pre-rhombomeres. Our single-cell RNA-seq analysis shows molecular evidence for pre-rhombomeres at 10 hours post fertilization (hpf) as well as identified distinct cell identities for each rhombomere at 13hpf in zebrafish. In our data, we find three clusters at 10hpf that we postulate correspond to r2/r3, r5/r6, and r4, respectively. These data show the first molecular definition for pre-rhombomeres, and we have verified these expression patterns *in vivo* using HCR RNA-FISH. Notably, we find that transcription factor Vestigial-Like 3 (Vgll3) has restricted expression at r2 at 13hpf but is coexpressed with egr2b, an r3 marker, in progenitor cells at 10hpf. We hypothesize that transcription factor Vgll3 is necessary for promoting rhombomere 2 cell fates and suppresses rhombomere 3 cell fates by disrupting egr2b's autoregulatory loop. To do so, embryos from the in-cross of our heterozygous Vgll3 mutants as well as our Vgll3 transgenic line will be screened for phenotypes in rhombomeres 2 and 3. Rhombomere phenotypes will be screened for using confocal imagery HCR RNA-FISH in fixed embryos at 10, 13, and 16 hpf, with probes labeling markers for rhombomeres 1 (irx1b), 2 (Vgll3), and 3 (egr2b). The width of the labeled rhombomeres will be measured using FIJI image processing software. Embryos will then be genotyped blind after imaging using the allele-specific genotyping method KASP. Funded by NIH #NS038183 and NIGMS #5T32GM136444-04.

1153F ***Dpp9* regulates lower jaw bone development and inflammasome activation in zebrafish** Sarah LaPotin, Madeleine Servais, Kristen M. Kwan Human Genetics, University of Utah

DPP9 deficiency is an autoinflammatory disease caused by loss of function mutations in DPP9. In this disease, there is typically persistent inflammasome activation and associated skin, craniofacial, and/or skeletal defects. The presence of developmental defects in patients with DPP9 deficiency suggests DPP9, in addition to functioning as a regulator of the immune system, could have an uncharacterized role in embryonic development.

In order to investigate the function of DPP9 in development, we generated stable *dpp9* mutant zebrafish. *Dpp9* mutants have craniofacial abnormalities and display a defect of a specific lower jaw bone. The affected bone, the anguloarticular, is highly reduced and misshapen in all homozygous mutants. Similar to wild type and heterozygous siblings, anguloarticular calcification begins in mutants at 7-8 dpf and starts to occur in the correct location. However, by 10 dpf and forward in developmental time, the anguloarticular is significantly shorter and smaller in mutants suggesting a defect impacting the subsequent bone growth and shaping rather than initiation. Interestingly, although cartilage defects frequently accompany lower jaw bone defects, the jaw cartilage elements appear normal in morphology in *dpp9* mutants, suggesting a defect specific to this bone formation.

Moving forward, a major goal is to uncover the cellular mechanisms underlying the anguloarticular defect in *dpp9* mutant zebrafish. Potential cellular mechanisms we are currently investigating include localized ectopic cell death and dysregulated osteoclast activity. We are also investigating the possibility of hyperproliferative cartilage or premature ossification of skeletal elements outside the jaw impacting the anguloarticular.

We also aim to determine the molecular mechanisms underlying this phenotype. Similar to humans with DPP9 deficiency, *dpp9* mutant zebrafish display inflammasome activation. We are investigating the spatial and temporal dynamics of

inflammasome activation in these fish, and if inflammasome is ultimately driving the anguloarticular phenotype. Additionally, as *dpp9* appears ubiquitously expressed, another goal is to determine how global loss of a ubiquitously expressed gene can cause what appears to be this specific defect of a lower jaw bone.

1154F **Cornea morphogenesis in normal development and in a model of Axenfeld-Rieger Syndrome** Emily D Woodruff, Ella S Habbeshaw, Kristen M Kwan Department of Human Genetics, University of Utah

The cornea is the transparent anterior-most tissue that protects the eye and refracts light entering the eye. Three main tissues comprise the cornea: the corneal epithelium (outer layer), corneal stroma (middle layer), and corneal endothelium (innermost layer). Proper formation of these three tissue layers during development is crucial for function; improper formation can lead to visual impairment, as seen in manifestations of a congenital genetic disorder, Axenfeld-Rieger Syndrome (ARS). ARS is characterized by structural defects in the anterior eye including the cornea, iris, and anterior chamber. Despite the importance of the cornea for vision, the cellular and molecular mechanisms underlying cornea formation in normal conditions and in ARS remain poorly understood.

We are investigating the cellular basis for cornea formation under normal conditions and in an ARS model, the *pitx2* mutant zebrafish. Our initial observations of the *pitx2* mutant zebrafish eye, which are consistent with previous reports of *pitx2* mutant and *pitx2* deficient zebrafish, revealed that *pitx2* mutant corneas lack the multiple cell layers seen in wild type, and the anterior chamber is reduced. The corneal endothelium could not be distinguished from the overlying epithelium, suggesting it may be reduced or possibly absent.

We asked whether cell proliferation or death might contribute to normal corneal development, and whether these processes might be disrupted in the *pitx2* mutant. In wild type embryos, we detected relatively few mitoses in the developing cornea from 30-120 hours post fertilization and very few apoptotic cells were observed. We next assayed cell proliferation and death in *pitx2* mutant embryos and found similar results compared to the wild type. This suggests that aberrant cell proliferation and apoptosis are unlikely to contribute to the mutant phenotype, and that other processes, such as cell migration, may be affected in the *pitx2* mutant, consistent with previous predictions.

Cranial neural crest cells, which express *pitx2*, migrate into the anterior eye and form the corneal endothelium, therefore, this cell population may be affected in the *pitx2* mutant, leading to defects in the anterior segment. We are currently testing this using multidimensional confocal imaging and 4D cell tracking using our custom software to track individual cells as they assemble to form the corneal endothelium in live zebrafish embryos.

1155F **Thyroid hormone regulates cell migration via PDGF signaling during post-embryonic zebrafish dermal development** August Carr, David Parichy, Andrew Aman Biology, University of Virginia

The dermis is an important component of vertebrate skin that provides mechanical strength and underlies patterning and morphogenesis of skin appendages. Zebrafish are a useful model system for discovering mechanisms that govern dermal development because we can easily visualize the entirety of this process in live animals while conditionally manipulating gene expression. We have shown that zebrafish dermal development requires migration of dermal progenitor cells from the larval hypodermis which subsequently differentiate as fibroblasts and papillary dermal cells. Our previous live imaging experiments showed that dermal progenitor migration spreads across the larval skin in a ventral to dorsal wave and is regulated by thyroid hormone. To further probe mechanisms that regulate thyroid hormone dependent migration, we used single cell RNA sequencing on skins from control and hypothyroid fish to analyze differential gene expression. Multiple transcripts were downregulated in hypothyroid skin and therefore represent potential thyroid hormone target genes. Among these candidates were genes encoding ligands in the PDGF (platelet derived growth factor) pathway. To test the role of PDGF signaling in dermal development, we performed conditional gain and loss of function analyses of PDGF pathway ligands and receptors. These experiments revealed that PDGF signaling is necessary and sufficient for dermal progenitor cell migration and differentiation, but not for skin appendage formation. Thyroid hormone has complicated effects on vertebrate skin development and homeostasis, and this work provides a novel mechanism connecting the hormone to cellular and molecular mechanisms of development.

1156F **Elucidating the Role of *Iroquois Transcription Factor 4a* in Kidney Development** Aisling L Kruger, Hannah Wesselman, Rebecca A Wingert Department of Biological Sciences, University of Notre Dame

Chronic kidney disease is a prominent problem in the US, impacting millions of Americans a year. In order to better address this issue, more advanced knowledge of kidney function and development is critical. The kidney is a vital organ responsible for both filtering waste from the blood, and balancing ion concentrations in the blood. These roles are carried out by the nephron, the functional unit of the kidney. Although nephron development is vital to renal function, there has been limited

research into the genetic mechanisms that regulate their formation. There are many genetic pathways that play crucial roles in the various components of kidney formation, one of which is the Iroquois (Irx) gene family of transcription factors. Of these, *Iroquois transcription factor 4a* (*irx4a*), is previously unstudied in regards to its role in nephrogenesis. The zebrafish is a useful model to study nephron development due to the high conservation in nephron composition with humans. Here, using whole mount in situ hybridization to assess spatiotemporal expression, we found that *irx4a* was expressed in the proximal straight tubule (PST) and distal early (DE) regions of the embryonic nephron. Interestingly, *irx4a* transcripts were first expressed at around the 12 somite stage (ss) in the renal progenitors which found these segment regions. Further *irx4a*+ cells exhibited a speckled expression pattern within the nephron, which suggests that it is likely a marker of either multiciliated cells (MCCs) or transporter cells. In the zebrafish kidney, MCCs are responsible for driving fluid flow through the kidney. We hypothesize that *irx4a* acts redundantly with *irx2a*, another member of the Iroquois gene family, because the two genes have very similar expression patterns. *irx2a* is expressed in MCCs, so it is believed that *irx4a* is as well. To examine whether *irx4a* is required for nephrogenesis, *irx4a* deficient embryos were created through the microinjection of a splice blocking morpholino. *irx4a* knockdown caused a significant decrease in the number of MCCs present in the nephron. This implies that *irx4a* plays a vital role in proper MCC formation. Future studies will examine the consequence of dual *irx4a*/*2a* deficiency on MCC ontogeny. Gaining insight into the function of genes such as *irx4a* will allow for greater understanding of how kidneys develop. This knowledge could provide critical insight into better understanding and eventually treating congenital and chronic kidney diseases.

1157F **Mural cells in an injured zebrafish heart change their identity to fibroblast-like cells and promote regeneration.** Subir Kapuria, Ching-Ling Lien Cardiothoracic Surgery, Children›s Hospital Los Angeles

Mural cells associate with the blood vessels on their abluminal surfaces and regulate vessel growth, stability, and contractility. Besides being a mere structural support and modulator of the vessels, mural cells also affect other surrounding cells during zebrafish heart regeneration. Previously we showed that, the mural cells express injury-responsive genes in the amputated hearts. Here we are showing not only do the mural cells change the gene expression but they also change their identity by expressing fibroblast marker genes and migrating away from the vessels toward the injury area. By Notch activity reporter, I observed that the mural cells on the injury surrounding blood vessels activate notch signaling and show this activity throughout the heart regeneration. Some of these notch-activated mural cells migrate toward the injury site away from the blood-vessels. By single-cell RNA sequencing analysis and confocal imaging of the mural cells in the injured hearts, I found that these mural cells express different fibroblast markers and genes that encode growth factors, angiogenic factors, extracellular matrix components, and cytoskeletal structure regulators what indicate their growth-promoting and migratory nature. Alltogether by changing their gene expression, identity, and cellular communications, the mural cells generate a conducive environment for heart regeneration. In this process, the mural cells interact with the immune cells including macrophages, endothelial cells, cortical cardiomyocytes, and epicardial cells. From this finding, the mural cells appear as the responsive, plastic cells which change their identity according to the surrounding need and a potential target for treating cardiovascular diseases.

1158S **Tissue-specific RNA-seq identifies genes governing male tail tip morphogenesis in *C. elegans*** Karin C Kiontke[1], Alyssa Woronik[2], Adam Mason[3], Antonio Herrera[4], David H. A. Fitch[5] [1]New York University, [2]Biology, Sacred Heart University, [3]Biology, Siena College, [4]Science, Baylor School, [5]Biology, New York University

The 4 tail tip cells (hyp 8-11) of *C. elegans* undergo male-specific changes in L4, making a round instead of pointed tail tip. A main regulator for this Tail Tip Morphogenesis (TTM) is the transcription factor DMD-3 (Mason et al. 2008). RNA-seq of laser-dissected early L4 tail tips identified 564 genes differentially expressed (DE) in wild-type (WT) males vs. *dmd-3(-)* males and hermaphrodites. To validate our data, we made transcriptional reporters for 40 DE genes. We found male tail tip-specific expression for 20 of these genes. Analysis of the RNA-seq data showed that: (1) The transcription profile of *dmd-3(-)* tail tips is similar to that in hermaphrodites. (2) Only 11 DE genes were also found in a whole-genome RNAi screen for defective TTM (Nelson et. al. 2011). (3) GO analysis of genes that are more highly expressed in WT male tail tips finds enrichment of terms that indicate high translational activity. (4) GO terms enriched for genes with reduced expression in WT males are consistent with these genes being involved in cuticle maintenance. (5) Only 4 collagens are upregulated in WT male tail tips; two of these, BLI-1 and COL-20, are found in the cytoplasm of the tail tip cells. (6) Several genes in the chondroitin synthesis pathway are DE. Their reporters are expressed in male tail tips, and knockdown results in a TTM phenotype. We hypothesize that a chondroitin proteoglycan is secreted into the space between male tail tip and L4 cuticle. FBN-1 is a candidate for the core protein. 39 transcription factors are DE, indicating that the gene-regulatory network downstream of DMD-3 is complex and potentially modular.

Using reporters for tail-tip-specific proteins identified in this study along with markers for cellular components (adherens junctions, extracellular matrix, cytoskeleton, etc.), we provide a detailed description of TTM and the fate of the tail tip cells.

We are developing a protein reporter toolkit to further study the subcellular events during TTM.

1159S **Integrating temporal, positional, and sex-specifying cues in *C. elegans* neurogenesis** Jennifer R Wolff[1], Emma Carlson[1], Jisoo Yeom[1], Andrea K Kalis[2] [1]Biology, Carleton College, [2]Biology, St. Catherine University

Development of neural circuits that control reproductive behaviors requires that neuronal precursors adopt programs of survival, division, and differentiation that are unique to each sex. We seek to understand how sex-determining signals are integrated with sex-shared programs of neural development, using the well-understood neuronal development of *C. elegans* as a model. In *C. elegans*, several neuroblasts of the P lineage divide in males, but not in hermaphrodites, initiating divergent programs of differentiation in the ventral cord and tail. These sex-specific programs depend on the sex determination factor TRA-1 and the Hox proteins MAB-5 and LIN-39. With our students at Carleton College and St. Catherine University, we have developed reagents to address how these regulators interact with each other and with unidentified factors to promote sex-specific nervous system organization. Notably, using a cell-cycle sensor, *mcm-4p::cdksensor::gfp*, we can directly observe neuroblast divisions that occur during sexual maturation to produce male-specific ventral cord and tail neurons relatively late in development, after the majority of sex-shared neurons have started differentiating.

One class of these "late-blooming" neuroblasts, the ventral cord Pn.aap neuroblasts, generates CA and CP neurons that influence mating movements and sperm transfer. Pn.aap neuroblasts divide in males to generate nine CA/CP neuron pairs, and either die or differentiate in hermaphrodites to produce six VC neurons, which modulate egg-laying. We have previously shown that differentiation, division, and survival of sex-specific ventral cord neurons depends on the coordinated activities of LIN-39 and MAB-5. We find that *mcm-4p::cdksensor::gfp* is expressed male-specifically in the ventral nerve cord beginning in late L2, with GFP expression becoming cytoplasmic in early-mid L3, indicating cell cycle commitment. The Pn.aap cells divide in mid-late L3 and continue to express GFP in both daughters through L4. Analysis of sex-specific cell cycle dynamics will allow us to investigate how LIN-39 and MAB-5 interact with cell cycle regulators and developmental timers to influence sex-specific fates of CA and CP neurons.

To further investigate sex-specific Pn.aap division, we have created a strain, P-masc, in which the P lineage has been selectively masculinized with an *hlh-3p::fem-3::mCherry* transgene. In XX P-masc worms, mCherry is expressed in all ventral cord neurons from L1 through adulthood. Strikingly, Pn.aap neuroblasts divide during L3, with posterior daughters differentiating as serotonergic neurons, a fate normally reserved for males. Further study of masculinized neurogenesis in the context of an otherwise female soma will shed light on the role of Hox proteins and other key regulators in both cell-autonomous and non-autonomous aspects of sex-specific neuronal development.

1160S **EGL-38/Pax controls cell type-specific matrix identity in the *C. elegans* vulva** Helen F Schmidt, Chelsea B Darwin, Meera V Sundaram Genetics, University of Pennsylvania

The development and function of tubular organs, including the lungs, intestines, and inner ear, depends in part on an apical extracellular matrix (aECM) that lines the organ interior. aECMs contain many components that are shared across organisms such as mucins, collagens, and zona pellucida domain (ZP) proteins. We are interested in how these components become organized into distinct spatial domains. Our lab found that in the *C. elegans* vulva, several matrix factors are associated with the surface of specific cell types despite being ubiquitously transcribed. Therefore, we hypothesize that each cell type expresses different matrix organizing factors that recruit its unique set of aECM proteins.

In the vulva, the two major cell types are determined by signaling - EGF-Ras-ERK specifies primary (1˚) cells (vulE/F) while Notch specifies secondary (2˚) cells (vulA/B1/B2/C/D). How downstream targets of signaling control the unique features of each cell type is less clear, as no "master regulator" transcription factors have been found. Since aECM is one marker of cell type identity, we focused on understanding what controls this feature.

We tested whether mutants in vulva-expressed transcription factors affected the localization of fluorescently tagged ZP proteins LET-653 and NOAH-1. Both proteins are broadly expressed in the vulva, but LET-653 assembles via its ZP domain (LET-653(ZP)) near the surface of 1˚ cells, while NOAH-1 is enriched in a matrix "spike" near the membrane of the 2˚ cell vulC. We found that the paired-box transcription factor EGL-38 is required to recruit LET-653(ZP) to the 1˚ cell matrix and exclude NOAH-1 from it. Consistent with the lab's previous result that LET-653(ZP) matrix assembly requires EGF-Ras-ERK specification of 1˚ cells, we found EGL-38 expression depends on the same signaling pathway. We propose that vulva cell identity is controlled by multiple transcription factors each driving gene regulatory sub-programs for distinct features of the cell type, and that EGL-38 controls the aECM sub-program of 1˚ cells. Our ongoing work is using single-nucleus RNA-Seq of sorted vulva nuclei to better understand the biology of the 7 different cell types and identify EGL-38 targets and candidate matrix organizers with cell type-specific expression.

1161S **SPN-4 promotes 3'UTR-dependent *lin-41* mRNA clearance during the oocyte-to-embryo transition in *Caenorhabditis elegans*** Karissa Coleman[1], Naly Torres[1], Caroline Spike[2], Tatsuya Tsukamoto[2], Micah Gearhart[2], Erika Tsukamoto[2], David Greenstein[2], Erin Nishimura[1] [1]Colorado State University, [2]University of Minnesota

Regulation of maternally inherited mRNA transcripts during the early stages of zygotic development drives the cellular divisions at the oocyte-to-embryo transition (OET). Many maternally inherited transcripts enriched in the oocyte are rapidly degraded after fertilization (Stoeckius et al., 2014) by associating with SPN-4, a cytoplasmic RNA-binding protein that likely recruits the Ccr4-Not deadenylase complex. Using immunopurification, we purified SPN-4 from late-stage oocytes and performed RNA-seq to identify 728 mRNAs that associate with this RNA-binding protein with 4-fold enrichment, $P<0.05$. Of these, *lin-41* mRNA was confirmed as a SPN-4 associated target. Indeed, single-molecule fluorescence *in situ* hybridization (smFISH) studies confirm that *lin-41* mRNA requires SPN-4 and its own 3'UTR for proper clearance in early embryos. To determine the sequence within the *lin-41* 3'UTR required for *lin-41* mRNA degradation in early embryos, we systematically deleted regions within the *lin-41* 3'UTR and performed smFISH to assess *lin-41* mRNA abundance. Using a FISHquant analysis pipeline, we observe deletions that remove a sequence within the FOX region of the *lin-41* 3'UTR significantly increase *lin-41* mRNA abundance. This result suggests this region recruits SPN-4 to promote mRNA clearance. We hypothesize other maternally inherited transcripts that contain potential SPN-4-binding sites also associate with SPN-4 to regulate their abundance and ultimately control cell fate during the OET.

1162S **Understanding the Machinery of *erm-1* mRNA Localization in *C. elegans* embryos: Implications for Local Translation and Cellular Regulation** Naly Torres[1], Karissa Coleman[2], Luis Aguilera de Lira[2], Brian Munsky[3], Erin Osborne Nishimura[2] [1]Colorado State University, [2]Biochemistry & Molecular Biology, Colorado State University, [3]Chemical & Biological Engineering, Colorado State University

The precise localization of maternally inherited mRNAs is crucial for establishing cell fate and body axis patterning during the oocyte-to-embryo transition (OET) in *Caenorhabditis elegans*. Because transcription is repressed at these stages, it is possible embryos rely on the localization of maternal mRNA transcripts for precise spatial and temporal control of gene expression and local translation. Indeed, the maternally inherited *erm-1* mRNA within early embryos localizes at plasma membranes (Parker et al., 2020) via translation-dependent localization (Winkenbach et al., 2022). In this study, we aim to understand the machinery of *erm-1*/ERM-1 localization to the plasma membrane, as ERM proteins play a pivotal role in connecting the inner cytoskeleton to the plasma membrane, contributing significantly to various physiological processes. Using a high-yield eggshell permeabilization strategy to impede microtubules (Nocodazole) or actin (Cytochalasin D) followed by single-molecule Fluorescence In Situ Hybridization (smFISH), we demonstrated that the loss of microtubules perturbs *erm-1* mRNA localization to the plasma membrane. Indeed, RNA interference knockdown studies confirm a role for KLP-14 kinesin in proper localization. To determine the significance of microtubule loss on *erm-1* transport, we used the FISHquant analysis pipeline to quantify the total *erm-1* molecules at the plasma membrane. This result suggests that *erm-1/*ERM-1 localization to the plasma membrane depends on microtubules and associated motors for localization, distinguishing between active and passive mechanisms. We hypothesize that translating *erm-1* molecules are transported via microtubules and anchored at the plasma membrane, while non-translating mRNAs remain in the cytoplasm.

1163S **Sexually dimorphic regulation of the PIWI/piRNA pathway impacts heat-induced male infertility in *C. elegans*** Nicole A Kurhanewicz[1], Acadia L DiNardo[1], Hannah R Wilson[1], Jessica A Kirshner[2], Margaret R Starostik[2], John K Kim[2], Diana Libuda[1] [1]Biology/IMB, University of Oregon, [2]Biology, Johns Hopkins University

The maintenance of genomic integrity during sperm and egg development is fundamental for fertility and proper genome inheritance across generations. Germ cells require precise regulation of gene expression to silence deleterious genomic elements, such as transposons, which can cause DNA damage and heritable mutations associated with both infertility and birth defects. During spermatocyte and oocyte development, the conserved PIWI/piRNA small RNA pathway represses transposon activity in part by adding the repressive chromatin mark H3K9Me3. Previous work in *Caenorhabditis elegans* demonstrated that acute heat exposure produces elevated DNA damage associated with transposon activity only in spermatocytes and not oocytes. Here our data indicate a role for the PIWI/piRNA pathway in the sexually dimorphic production of heat-induced DNA damage and Tc1-Mariner family transposon activity in spermatocytes. Using immunofluorescence imaging of PIWI/piRNA pathway master regulator PRG-1, we identify sexually dimorphic and heat-sensitive localization and morphology of PRG-1 within the perinuclear nuage structures of developing germ cells. Further, we reveal that conditional knockdown of PRG-1 knockdown enhances heat-induced DNA damage only in developing sperm. Using chromatin immunoprecipitation of the PIWI/piRNA-related repressive chromatin mark H3K9Me3, we find heat shocked adult hermaphrodites undergoing oogenesis experience a general increase in the repressive chromatin mark H3K9Me3, while H3K9Me3 is largely unaffected in heat shocked males undergoing spermatogenesis. Notably, we observe that heat shock specifically increases H3K9me3 at the DNA transposon Tc1 in adult hermaphrodites. Commensurate with increased H3K9Me3 in adult hermaphrodites, our RNAseq

results also demonstrate heat produces a global repression of transcription in adult hermaphrodites. In contrast, males only exhibit a slight increase in global gene expression. Moreover, we find three-times as many PIWI/piRNA pathway targets are differentially expressed by heat shock in hermaphrodites compared to males. Taken together, these data indicate oocytes have a robust, gene-repressive response to heat that spermatocytes lack. This work provides insight into fundamental differences between the oogenic and spermatogenic developmental and genome maintenance programs, and the broader mechanisms underlying the heat-induced male infertility.

1164S **Roles for fate specifying transcription factors in collective cell migrations and fate transformations in *C. elegans* embryogenesis** Prativa Amom[1], Breana D Anderson[1], Tushar H Ganjawala[1], Radmehr Molaei[1], Jorin T Hanson[1], Amanda L Zacharias[2,3] [1]Developmental Biology, Cincinnati Children's Research Foundation, [2]Developmental Biology, Cincinnati Children's Research Foundation, [3]Pediatrics, University of CIncinnati College of Medicine

In vertebrates, cell fate specification is directly linked with collective cell migrations. In *C. elegans*, the role for lineage determining factors is established in gastrulation, but the role for fate specifying transcription factors in gastrulation and other collective cell movements including ventral cleft closure remained unknown until recently. We previously evaluated the roles of the transcription factors (TFs) that specify neuronal, muscle, skin, and pharyngeal fates to determine their roles in collective cell movements. We found that the skin TFs *elt-1* and *nhr-25* are required to promote mesoderm gastrulation, pharynx organization, and ventral cleft closure, while both muscle fate TFs *hlh-1* and *unc-120* also promote ventral cleft closure. To determine whether fate transformations played a role in disrupting these collective cell movements, we examined the expression of various cell fate markers in mutant embryos. In particular, we wanted to test the hypothesis that cells would express the fate markers of "sister" lineages, since their shared history suggests that they might have the factors necessary to activate these genes.

We found in *nhr-25(jm2389)* mutant embryos, some Caa and Cpa daughter cells that would normally adopt hypodermal fates activate an unc-120 promoter reporter, indicating they have activated the muscle fate of their Cap and Cpp cousin cells. This is consistent with previous reports that utilized RNAi knockdown and microarray to evaluate gene expression. Conversely, in *hlh-1(cc561)* mutant embryos treated with RNAi against *unc-120*, the MSap and MSpp daughters that normally produce muscle do not activate a PHA-4::GFP fosmid reporter, a marker of pharyngeal fate, which is expressed by their MSaa and MSpa cousin cells. These results suggest that different lineages have evolved distinct mechanisms for fate specification. While our findings are consistent with previous reports that the TFs specifying muscle and skin fates are mutally antagonistic in the C lineage, they also suggest that the mechanism(s) for establishing expression of muscle and pharyngeal fate TFs in distinct sister lineages of MS are more complex.

1165S **Exploring the role of *C. elegans* furin proteases in the cleavage of ZP proteins** Chelsea Darwin, Helen Schmidt, Susanna Birnbaum, Meera Sundaram Genetics, University of Pennsylvania

Zona Pellucida (ZP) domain proteins are a highly conserved family of glycoproteins first characterized in the coating of mammalian egg cells. In *C. elegans*, these proteins are important structural components of the precuticle apical extracellular matrix (aECM). When these proteins do not localize to their correct place in the aECM, it results in structural defects in developing organs. Many ZP proteins are cleaved at a dibasic site that resembles the consensus for furin proteases, and we have shown this cleavage to be essential for function and correct localization to the matrix. However, the protease(s) responsible for cleavage of most ZP proteins, in C. elegans as well as mammals, have not yet been identified.

C. elegans has 4 furin proteases: BLI-4, KPC-1, EGL-3, and AEX-5. We recently showed that BLI-4 promotes collagen secretion and cuticle assembly in the embryo. However, despite their dramatic cuticle defects, *bli-4* null mutants still had normal localization of ZP proteins in the embryo pre-cuticle.

Here we used the developing *C. elegans* vulva as a model to investigate the *C. elegans* furins and their relationship to the ZP proteins LET-653 and NOAH-1. Of the four furins, KPC-1 and BLI-4 have been identified as expressed in vulval cells. Our data from *kpc-1* and *egl-3* null mutants, *bli-4* isoform specific knockouts, and *aex-5* reduced function mutants, suggest none of the furins affect the localization of either ZP protein. However, *bli-4* null mutations are lethal, so we have not yet been able to visualize gene knockout in the vulva. Future directions for this project include looking at the phenotypes of double mutants, and screens of other candidate proteases outside of the furin family.

1166S **tab-1, a C. elegans ortholog of BSX, is required for the normal development of the ABalaaa lineage** William Marchese, Eli Preston, Ella Eseigbe, Alexis Garcia, John Murray University of Pennsylvania

The availability of genome-wide lineage-resolved expression atlases and the robustness of C. elegans embryonic cell lineages

create the potential to systematically identify regulators of each sublineage. Here we focus on the ABala lineage, which primarily produces neurons and glia, and for which mechanisms controlling most lineage decisions are unknown.

We screened loss of function mutants of five transcription factors identified in expression databases as expressed in ABala or its sublineages in an attempt to find TFs required for normal ABala development. Using 4-D confocal microscopy and Starrynight cell lineage tracing software, we identified defects in cell division timing, cell death patterns, downstream gene expression or cell positions within the ABala lineage in several transcription factor mutants, including ceh-32, ceh-37 and tab-1.

We focused in more detail on the role of tab-1, since a tab-1 mutant had the most striking impact on division patterns, causing ectopic deaths in the of ABalaaa lineage consistent with an ABalaaa->ABalaap lineage transformation. tab-1 mutants had additional defects in other non-ABala lineages that express TAB-1. This further suggests that tab-1 is responsible for patterning the ABala downstream lineages. Past work from other labs showed that tab-1 is required to generate the ABalaaa-derived neurons AVD, and AIN. We tested whether the ILsoL and ILsoR glia, which are also ABalaaa-derived, are defective in tab-1 mutants and found that they are absent, suggesting tab-1's role in ABalaaa development is not neuron-specific.

To identify potential upstream regulators of tab-1, we explored the upstream regulatory region of tab-1. Using available ChIP-seq and ATAC-seq data we identified two candidate enhancer regions and are currently testing these regions for enhancer activity. We depleted candidate upstream regulators of tab-1 and found that RNAi depletion of ceh-37/Otx causes partially penetrant loss of tab-1::GFP expression.

Future work to better characterize the function of tab-1 in shaping elegans embryonic development will require us to test for the necessity of these modular enhancers by CRISPR deletion of the proposed sites, to test for phenotypic impacts on the embryonic lineage and to identify targets of tab-1 in ABalaaa sublineages. Overall, this work identifies novel functions for a conserved homeodomain TF and supports the feasibility of using large scale imaging and scRNA-seq atlases to identify lineage regulators.

1167S **Sense organ glia secrete extracellular matrix proteins customized for different types of sensory cilia** Wendy Fung[1], Taralyn Tan[1], Irina Kolotuev[2], Maxwell Heiman[1] [1]Boston Children's Hospital, Harvard Medical School, [2]University of Lausanne

We perceive the world through sense organs containing sensory cells with specialized cilia that detect touch, smell, taste, or other stimuli. Importantly, all sense organs are covered with an apical extracellular matrix (aECM) that serves as the interface between cilia and the environment. Electron microscopy studies have revealed an extensive diversity of aECM structures that are customized to sense organ function, from force transducers for touch to nanoscale pores for taste. However, while cilia are well studied, the aECM structures that cover them have been largely ignored. *C. elegans* offers an ideal system for determining the molecular basis of aECM differences at each sense organ. In addition to the major amphid sense organs, the *C. elegans* nose contains 16 minor sense organs with a single mechanosensory neuron (OL; hermaphrodite CEP) or paired mechanosensory and chemosensory neurons (IL; male CEP). In all cases, the ciliated endings of the sensory neurons protrude through a tube formed by two glia, called the sheath and socket. The socket glial cell forms the distal portion of the tube, makes junctions with the skin, and contributes to the cuticle, an aECM body covering. We find that the socket glia of each sense organ (OLso, ILso, CEPso) express different aECM proteins that localize to discrete extracellular domains around specific cilia. For example, using endogenous tags, we find that the collagen COL-56 is expressed exclusively by OLso and CEPso glia and localizes in an extracellular disc over mechanosensory cilia, possibly contributing to force transduction. By contrast, the collagen COL-53 is expressed exclusively by ILso and male CEPso and localizes to the pore through which chemosensory cilia access the environment. Interestingly, we find that COL-53, COL-177, and GRL-18 – a novel aECM protein that we find localizes to transient nanoscale rings during cuticle synthesis – are expressed in male, but not hermaphrodite, CEPso glia. Using genetic screens, we find that this sex-specific difference in CEPso glia is controlled by the known sex identity regulators *mab-3*, *lep-2*, and *lep-5*, as well as novel regulators *nfya-1*, *bed-3*, and *jmjd-3.1*. Finally, by manipulating sex-specific gene expression in CEPso, we find that this switch is necessary and sufficient to pattern the overlying aECM into a nanoscale pore that accommodates the cilium of a male-specific chemosensory neuron. Together, our results suggest that glia secrete modules of aECM components that are customized for different cilia types and that define the interface between each cilium and its environment.

1168S **Understanding how cell fate coordinates localization of myosin activating machinery to drive cell shape changes** Taylor N Medwig-Kinney, Pu Zhang, Bob Goldstein UNC Chapel Hill

Apical constriction is a cell shape change resulting from contraction of actomyosin networks at the cell apex. Apical constriction plays a key role in development of the brain and spinal cord, failure of which is associated with neural tube defects. Polarized contraction of actomyosin networks can also drive cell extrusion, which is required for epithelial homeostasis and can contribute to tumorigenesis and metastasis when dysregulated. Despite the importance of precise coordination

of cell shape changes, how localization of myosin activating machinery is informed by cell fate and cell polarity is not well understood. Internalization of two endoderm precursor cells during *C. elegans* gastrulation provides a visually amenable and genetically tractable model to study apical constriction *in vivo*. Previous work from our group has shown that the myotonic dystrophy-related Cdc42-binding kinase MRCK-1 is a critical regulator of *C. elegans* endoderm internalization. MRCK-1 localizes to the apical surface of the endoderm precursors where it activates actomyosin contractility. Here, we quantify localization of endogenously-tagged myosin (NMY-2) and MRCK-1, and show that they both localize throughout the apical cortex. Surprisingly, we find that MRCK-1's localization pattern is distinct from that of another known CDC-42 interacting protein, WSP-1 (WASP) during apical constriction. We are now developing tools to determine if localization of MRCK-1 is sufficient to induce cell shape change and are investigating the mechanisms downstream of cell fate that coordinate its localization. This research will help draw long-sought connections between developmental patterning mechanisms and force generation.

1169S **The molecular interactions within the fertilization synapse in *C. elegans*** Xue Mei St. John›s University

Fertilization is a culminating event during sexual reproduction. Fertilization involves species-specific interactions between the gametes, including recognition, adhesion, and fusion, to form a zygote. The molecular mechanisms of the interactions are not well understood. Proteins on the surface of the gametes are thought to form complexes that mediate the interactions and eventually bring about fusion, and we call these complexes the fertilization synapse. In *C. elegans*, SPE-51 is one of the sperm secreted molecules that are required for fertilization. Loss of spe-51 results in normal-looking sperm that can migrate to the spermatheca but cannot fertilize the egg. The SPE-51 protein stays associated with spermatozoa, suggesting it is being tethered to the membrane by other molecules. Here, we examine the localization of SPE-51 in the absence of other sperm-surface fertilization molecules. Our preliminary data suggest that SPE-51 localization on the pseudopod is lost in the absence of SPE-38 but not SPE-9. Our findings will provide insights into the assembly and dynamics of the fertilization synapse.

1170S **Mutations affecting the genetic regulation of sperm activation give rise to infertility in the nematode *C. elegans*** Emily K Mincher[1], Allison Ramz[2], Benjamin Khaim[3], Xue Mei[3] [1]Biology, St. John's University, [2]Biology, St. Johns University, [3]Biology, St Johns University

Sperm activation in *C. elegans* is a post-meiotic differentiation process of efficient fertility, in which round spermatids are morphologically and functionally transformed into motile spermatozoa with pseudopods. Initiation of spermiogenesis is known to be genetically regulated by the poorly understood SPE-8 and TRY-5 pathways. Males can use both pathways, while hermaphrodites opt for the SPE-8 pathway. TRY-5 is a protease implicated in extracellular signaling in spermiogenesis. SPE-8 is an intracellular kinase that responds to an unknown activator. These semi-redundant pathways are associated with proteases, cell surface receptors, and intracellular signaling molecules, but other unidentified molecules are believed to be involved. With the use of forward genetic screening, we isolated a temperature-sensitive mutant allele *as47*. Hermaphrodites with the *as47* allele are self-sterile. This infertility defect can be rescued by mating *as47* hermaphrodites with wildtype males, suggesting this defect is sperm-related. Imagin under differential interference contrast (DIC) microscopy showed similar structures of the germline and spermatheca when compared to the control. Mutant hermaphrodites lay unfertilized oocytes. DAPI staining revealed that mutant hermaphrodite sperm were unable to migrate bac into the spermatheca, suggesting a fault in sperm motility. Hermaphrodite dissection showed that sperm were not fully activated *in vivo* in comparison to control sperm. Whole-genome sequencing identified a candidate gene of *as47* with a *tm5344* deletion. These two mutations fail to complement each other, suggesting they are involved in the same gene. The *tm5344* allele was hypothesized to give rise to sterility in *C. elegans*, similar to the *as47* allele. DIC imaging and DAPI staining of the *tm5344* hermaphrodites showed similar results to that of the *as47* allele. A strain called SJM5 was built containing the *tm5344* and *him-5* alleles to better study male fertility. Initial brood counts revealed a low incidence of fertility among these mutant males. We will investigate sperm activation of SJM5 males with *in vitro* sperm activation assays using Pronase, zinc, and zinc ionophore. The gene of interest, implicated in both alleles, encodes a Ser/Thr phosphatase that is most similar to that of the PP1 phosphatase. The goal of this work is to determine if this gene may be part of the SPE-8 or TRY-5 pathways that regulate the initiation of sperm activation.

1171S **LIN-35, the DREAM complex, and inactivation of CED-9 function to promote germline apoptosis under moderate temperature stress in *C. elegans*** Margaret N Davidson[1], Frances V Compere[2], Kristen A Quaglia[1], Lisa N Petrella[1] [1]Biological Sciences, Marquette University, [2]Syracuse University

In *C. elegans*, germline apoptosis occurs regularly to produce healthy oocytes. However, elevated temperature stress along with other stressors leads to an increased rate of apoptosis in the germline, which is likely beneficial for preserving fertility. Previous work has shown that LIN-35 promotes stress-induced apoptosis through regulation of *ced-9* expression. CED-9 protein generally is inactivated in order to promote apoptosis. LIN-35 cannot directly bind to DNA but can regulate gene expression as a member of the DREAM complex. Here, we wanted to determine if LIN-35 and DREAM complex proteins play a role in promoting increased apoptosis in the germline when exposed during moderate temperature stress. We found that

in *lin-35* mutants and two DREAM complex mutants, *lin-54* and *lin-37*, germline apoptosis had a lower level of induction when compared with wild type in temperature stressed animals. We next wanted to investigate whether inactivation of CED-9 function was necessary to induce increased apoptosis during temperature stress. We found that in a constitutively active *ced-9* mutant, germline apoptosis had a lower level of induction when compared with wild type in temperature stressed animals, similar to what was seen in *lin-35* and DREAM complex mutants. Finally, we explored if decreasing the rate of apoptosis during temperature stress would lead to changes in cytoplasmic streaming, oocyte size or ovulation rates. We found that while wild type increased the cytoplasmic streaming rate during temperature stress, neither *lin-35* nor *lin-54* mutants increased their cytoplasmic steaming rate during temperature stress. In addition, *lin-35* and *lin-54* made fewer oocytes than wild type irrespective of temperature treatment. Finally, there was no clear temperature effect on ovulation rate in any strain, including wild type, although *lin-35* and *lin-54* did show a lower number of ovulation events than wild type irrespective of temperature treatment. Overall, our data points towards a model where CED-9 is regulated, maybe both at the mRNA expression level and the protein activation level, to activate increased levels of apoptosis during temperature stress. This activation is, in part, dependent upon LIN-35 and the DREAM complex and results in changes in cytoplasmic streaming and oocyte size and number.

1172S **Functional study of an alternative protein encoded by the dual coding gene *ZYX/zyx-1* with implications for dystrophinopathies** Noémie Frébault[1], Lise Rivollet[1], Benoît Grondin[1], Benoît Vanderperre[1], Claire Bénard[1,2] [1]Dept. Biological Sciences, CERMO-FC Research Center, Université du Québec à Montréal, [2]Dept. Neurobiology, University of Massachusetts Chan Medical School

Translation of alternative ORFs results in the synthesis of at least 30 000 “alternative proteins”. Some of these have been found to physically interact with the canonical protein encoded by the same gene, modulating their expression, cellular localization, and molecular function. Other alternative proteins can function independently of the canonical protein. While alternative proteins tremendously enrich the human proteome, very few have been studied to date. The conserved *ZYX/zyx-1* gene encodes not only the canonical protein Zyxin/ZYX-1 but also -as revealed by mass spectrometry studies in humans and *C. elegans*- an abundant and conserved alternative protein AltZyxin, which is produced from an alternative ORF. The gene *zyx-1* is required for synapse maintenance and dystrophin-dependent muscle degeneration in worms. Moreover, either overexpression or loss of *zyx-1* ameliorates the muscle degeneration phenotype caused by dystrophin deficiency, establishing *ZYX/zyx-1* as a promising therapeutic target for Duchenne muscular dystrophy. Canonical Zyxin is part of adhesion complexes, but the function of AltZyxin is unknown. To decipher the mechanism by which the gene *ZYX/zyx-1* regulates synapse maintenance and muscular degeneration, we investigate the function of the alternative protein AltZyxin in synapse and muscle biology in *C. elegans.* For this, we have generated CRISPR-engineered mutations to specifically disrupt either of the two encoded proteins, and determine the behavioral and anatomical consequences. Neuron- and muscle-specific rescue assays, localization studies, and an *in vivo* TurboID screen to identify AltZyxin interactors will inform on the functions and interplay between Zyxin and AltZyxin.

1173S **Regulated endoplasmic reticulum remodeling inhibits ectopic RNP condensates in oocytes** Jennifer A Schisa, Mingze A Gao, Nicholas A Trombley, Christya A Haddad, Cora A Zoet, Chloe A Munderloh, Victoria A Tice, Ashley A Cichon Biology, Central Michigan University

Infertility is a significant issue among women due to poor oocyte quality; however, factors affecting oocyte quality remain incompletely understood. Our long-term goal is to determine the extent to which the dysregulation of RNA binding protein (RBP) phase transitions contributes to infertility. We are currently focused on dissecting the regulatory pathways that modulate RBP phase transitions during oogenesis and maturation in *C. elegans*. Distinct subunits of the oligomeric CCT chaperonin promote phase separation of three different RBPs in meiotically-arrested oocytes and embryos. We asked if CCT modulates phase transitions and maturing oocytes, and because CCT subunits can function as monomers or an oligomer, we also asked if all subunits are required. We find depletion of an individual CCT chaperonin subunit is sufficient to result in ectopic condensates of MEX-3 and CAR-1/Lsm14 during maturation. These data are the first demonstration of CCT inhibiting RBPs during oogenesis and suggest the CCT chaperonin complex functions to maintain RBP phases during maturation. Because obligate substrates of CCT include actin and tubulin, we asked if *cct* condensation phenotypes are mediated by either cytoskeleton system. Upon depletion of *act-4*, we detected ectopic MEX-3 and CAR-1 condensates, suggesting actin may mediate the *cct(RNAi)* phenotypes. Actin regulates the endoplasmic reticulum in multiple developmental contexts, and the ER undergoes remodeling concomitant with phase transitions in arrested oocytes. Therefore, we hypothesized that CCT and actin modulate RBP condensation via modulation of ER structure. Strikingly, we found that depletion of *cct* or *actin* results in ectopic ER remodeling into sheet-like structures, consistent with the possibility that increased ER surface area promotes ectopic condensation of RBPs. Lastly, we determined the mRNA target of CAR-1, *spn-4*, appears to be translationally derepressed when ectopic CAR-1 condensates form in maturing oocytes. Ultimately, we hope to unravel the complex regulation of RBP condensation during oogenesis and maturation, and the role of regulated RBP condensation on maintaining oocyte quality.

1174S **Novel quasi-living cell morphology in the absence of EOR-1/PLZF, Chromatin Regulators, and WAH-1/AIF1 in a cell that dies via a non-canonical apoptotic program** Nathan Rather, Karen Juanez, Rashna Sharmin, Aladin Elkhalil, Piya Ghose Biology, University of Texas at Arlington

Programmed cell death (PCD) is a genetically programmed and evolutionarily conserved form of cell elimination required for normal development and homeostasis, with important implications for responses to cancer therapies.

We discovered a non-canonical apoptotic developmental cell death program in the *C. elegans* tail spike cell (TSC) called Compartmentalized Cell Elimination (CCE). During CCE the soma (cell body), proximal process, and distal process segments of this specialized cell die differently.

A forward genetic screen for CCE defects recovered a mutant, *ns957,* that shows a persisting enlarged soma alone with nuclear elevation. Genetic analysis of *ns957* revealed that the causative lesion is a mutation in the *eor-1* gene. Here we report a novel role for the EOR-1 transcription factor as well as it›s binding partner EOR-2, the chromatin modifiers MAU-2/MAU2 and SWSN-1/SMARCC1 in CCE. Interestingly, EOR-1 has been implicated in other forms of cell death although its mechanism remains unknown. Importantly, we find that caspase protease activity is normal in *eor-1*(-), suggesting that EOR-1 acts downstream of caspase in CCE.

We probed into the morphology of the remaining cell body on these mutants by generating an *eor-1* and *ced-12* (cell corpse engulfment gene) double mutant to determine if the persisting soma is a dead, but uncleared, apoptotic corpse. Interestingly, the soma remnant of this double mutant does not appear as a refractile cell, nor does it appear as a living cell, suggesting that it is not a persisting apoptotic corpse, but perhaps a quasi-living cell.

Using a candidate gene approach based on putative EOR-1 transcriptional targets we found that mutants for *wah-1*, which encodes a mitochondrial factor homologous to mammalian AIF1, phenocopy *eor-1(-)* soma phenotype. Interestingly, we also find fragmented mitochondria in the *eor-1(-)* persisting soma. Calcium signaling has been linked to cell death as well as the regulation of mammalian AIF1. We also found altered Calcium dynamics during CCE in *eor-1(-)*. Taken together, this suggests a link between EOR-1 regulated WAH-1, mitochondrial dynamics, and Calcium signaling in CCE.

Our study introduces a previously unreported life-to-death cell transition state that takes place during the novel cell death program of CCE. We also describe possible roles of genes implicated in cancer biology and other forms of cell death, as such our study may be highly relevant to developing cancer therapy.

1175S **The regulation of PP1 phosphatase in *C. elegans* sperm development and post-fertilization signalling** Shreyosi Bose, Martin Srayko Biological Sciences, University of Alberta

The fusion of gametic cells is a fundamental process that ensures the survival and propagation of sexually reproducing species. Sperm and oocytes are produced in the male and female germline, respectively, via meiotic divisions. The fusion of sperm and oocyte (fertilization) gives rise to the diploid zygote, which divides mitotically to form a multicellular embryo. Fertilization involves complex signalling pathways and strict regulation of the cell cycle. In most metazoans, oocytes arrest at multiple stages of meiosis to coordinate maturation with fertilization. In vertebrates, oocytes arrest at metaphase of meiosis II, and only complete the MII division after fertilization. This indicates that sperm-derived signals trigger female meiosis completion, though little is known about the molecular mechanism. Using the *C. elegans* hermaphrodite model system, the Srayko lab identified *memi-1/2/3* (meiosis to mitosis), oocyte-specific genes required for completion of female meiosis after fertilization. Loss of *memi* function causes embryonic lethality due to a “skipped MII” phenotype. Since this phenotype is also observed when a mature oocyte is not fertilized, the MEMI proteins likely represent a maternal component of the sperm-derived post-fertilization signal required for the proper meiosis-to-mitosis transition. In contrast to the *memi* loss-of-function phenotype, a gain-of-function mutation, *memi-1(sb41)* demonstrates entry into MII but a failure to exit MII. Genetic screens for *memi-1(sb41)* suppressors identified two nearly identical sperm-specific PP1 phosphatases, GSP-3/4. GSP-3/4 have been previously implicated in sperm meiosis and motility, but we found that these PP1s also contribute to the female MII program in the fertilized oocyte. Our suppressor screen also revealed a putative regulatory subunit of PP1 phosphatase. A FLAG-tagged version of this regulatory protein localized to the plasma membrane of activated sperm. Interestingly, this localization was restricted to the sperm cell body membrane, rather than the pseudopod, suggesting a model for how PP1 activity could be spatially regulated within crawling sperm. In fertilized embryos, the protein was tightly restricted to a zone around the condensed sperm DNA. A molecular and cellular characterization of this gene will be presented, as well as a model for its role in post-fertilization signalling.

1176S **An EMS screen for suppressors of *C. elegans memi-1(sb41)* reveals oocyte and sperm-specific pathways required for proper post-fertilization events** Rim Daher, Shreyosi Bose, Ish Jain, Rudra Banerjee, Jens Herzog, Caitlin Slomp, Martin

Srayko Biological Sciences, University of Alberta

Before fertilization, the *C. elegans* oocyte responds to an external, sperm-derived signal provided by major sperm protein (MSP). This signal releases the oocyte from its arrest, initiating the meiosis I division (MI). Normally a sperm enters the oocyte within a few minutes, but if fertilization does not occur, the MSP-stimulated oocyte aborts anaphase I, skips meiosis II (MII), and enters mitosis. This indicates that a second signal is provided upon sperm entry to specify the MII program. The paralogs, *memi-1*, *2,* and *3* (meiosis-to-mitosis transition) encode oocyte proteins that likely represent maternal components of this post-fertilization signal, because loss of all three paralogs also results in a skipped-MII phenotype. In contrast to the loss-of-function phenotype, a gain-of-function mutation, *memi-1(sb41ts),* results in fertilized embryos that complete MI, enter MII, but do not exit MII properly. In a previous RNAi screen, we identified a *gsp-3/4* as a suppressor of *memi-1(sb41)*. These genes encode sperm-specific PP1 phosphatases required for spermatogenesis and sperm motility (pseudopod treadmilling), but their involvement in the suppression of maternal *memi-1(sb41)* suggested that they also represent part of the sperm-derived signal.

A subsequent EMS-based screen recovered both intragenic and extragenic suppressors of *memi-1(sb41)*. Consistent with results from the RNAi-based screen, the EMS screen identified 4 alleles of *gsp-4*. In addition, the screen identified sperm-specific GSK3 kinases, *gskl-1* and *gskl-2*, which seem to act within the GSP-3/4 pathway. For example, like *gsp-3/4*, these genes are required for spermatogenesis and sperm motility via pseudopod treadmilling. In addition, we found that *gskl-1* and *gskl-2* cooperate with *gsp-3/4* for post-fertilization functions that involve MEMI. Using CRISPR gene editing, we have now confirmed most of the remaining extragenic suppressor mutations from this screen. Interestingly, these suppressors fall into two groups: 1) genes required for sperm-specific functions, which likely represent some component of the post-fertilization signal, or 2) maternal genes that function as genetic activators of *memi,* and thus, could modulate MEMI activity or levels within the fertilized oocyte. Current progress on the intragenic and extragenic suppressors will be presented.

1177S **The Transcription Factors PAL-1 and SEX-1 drive Ventral Nerve Cord Assembly in *C. elegans*** Nathaniel Noblett[1,2], Tony Roenspies[2], Stephane Flibotte[3], Antonio Colavita[1,2] [1]University of Ottawa, [2]Ottawa Hospital Research Institute, [3]University of British Columbia

The development of complex tissues requires the regulation of coordinated cell movements, including convergent-extension (CE). Common among processes that involve CE, including gastrulation and neural tube development, cells undergo coordinated intercalations that narrow and elongate the tissue. These movements require complex actomyosin organization and are facilitated by the formation and resolution of multicellular rosettes. We have previously shown that aspects of rosette-mediated CE are conserved within the embryonic *C. elegans* nervous system, where the process regulates assembly of the ventral nerve cord (VNC). During embryonic development, VNC assembly involves the intercalation of DA, DB and DD neurons at the midline to form a single nerve tract consisting of a stereotypical pattern and spacing of neurons. A genetic screen for VNC assembly defects identified PAL-1, a homolog of the CDX family, and SEX-1, a nuclear hormone receptor, as important players in VNC assembly. Mutation in these genes results in worms with anterior displacement and mis-ordering of VNC neurons at hatching, suggesting that VNC assembly in the embryo may be abnormal. We used timelapse microscopy to visualize VNC development in live embryos and assessed terminal neuron positions in larvae. New alleles of *pal-1* and *sex-1* were also generated using CRISPR-Cas9. We observed defects in *pal-1* mutant embryos that are characteristic of disrupted CE, including rosette resolution delays. These defects are independent of cell fate changes previously described in *pal-1* mutants, as the number of VNC neurons and terminal cell fate markers are normal. Additionally, we knocked GFP into the endogenous *pal-1* and *sex-1* loci. PAL-1::GFP is expressed in a number of posterior embryonic cell lineages, including a subset DA, DB and DD neurons. We found that point mutations and a small deletion approximately 10kb upstream of the *pal-1* start site resulted in loss of PAL-1::GFP expression in DA and DD neurons. *sex-1* is widely expressed during early embryogenesis but less so after the comma stage. Interestingly, *sex-1* mutants result in a similar loss of PAL-1 expression in DA and DD neurons suggesting that *sex-1* regulates *pal-1* transcription. Both *pal-1* and *sex-1* mutants display similar VNC neuron spacing defects at hatching. Together, this data highlights a role for the transcription factors PAL-1 and SEX-1 during VNC assembly and serve as the basis for further study into their regulatory targets.

1178S **DUO-1 plays multiple roles in meiotic prophase chromosome dynamics** Charlotte P Choi, Liesl Strand, Anne Villeneuve Developmental Biology, Stanford University School of Medicine

Meiosis is a crucial developmental process that generates haploid gametes from diploid germ cells, an essential feature of reproduction in eukaryotes. During prophase of meiosis I, germ cells undergo multiple major state transitions, involving massive remodeling of chromosome architecture and nuclear organization, without any intervening cell divisions. This dynamic program of changes in chromosome architecture involves a complex series of associations and dissociations of multiple different protein complexes that drive assembly, remodeling, and disassembly of meiosis-specific chromosome structures and dramatic changes in chromosome compaction. Failure to properly coordinate these processes can result in improper

segregation of chromosomes, resulting in aneuploid gametes and inviable zygotes. We are investigating the roles of DUO-1, a putative deubiquitinating enzyme, in mediating these dynamic chromosomal events of meiotic prophase.

We identified two mutations in *duo-1* in our recent "Green eggs & Him 2.0" screen for mutants defective in meiotic chromosome segregation, implicating DUO-1 as an important component of the meiotic machinery. *duo-1* mutants were notable in exhibiting highly decompacted chromatin at the end of meiotic prophase when chromosomes normally condense into compact bivalents in preparation for the meiotic divisions. Immunofluorescence analyses of *duo-1* null mutants indicate that DUO-1 is required for normal appearance of meiotic chromosome axes and synaptonemal complexes (SCs), for timely completion of meiotic DNA repair, and for late-prophase chromosome compaction. Further, by using a dual-AID-tagged allele to deplete DUO-1 for different durations during meiotic development, we were able to demonstrate distinct roles for DUO-1 in both assembly and stability of axes and SCs, indicating that the SC is a structure that requires active maintenance. Moreover, we demonstrated a separate and independent role for DUO-1 in promoting chromosome compaction at the end of meiotic prophase. Consistent with multiple roles in chromosome dynamics during meiotic development, 3xFLAG::DUO-1 and DUO-1::GFP localize predominantly in the nucleoplasm of meiotic prophase germ cells, increasing markedly in abundance during prophase progression. We are currently pursuing partners and targets of DUO-1 using the GFPnanobody::TurboID system. We are optimistic about this approach based on preliminary results showing that DUO-1::GFP is sufficient to direct Nanobody::TurboID to the nucleus.

1179S **Exploring the Complexity of the *kayak* Locus in Development** Juan R Riesgo-Escovar[1], Manuel Alejandro Zúniga-García[2] [1]Neruobiología del desarrollo y Neurofisiología, Universidad Nacional Autónoma de México, [2]Universidad Nacional Autónoma de México

The *kayak* locus consists of the gene '*kayak'* alongside two intronic genes— an uncharacterized long non-coding RNA and a predicted serine/threonine phosphatase. Through the generation of mutant clones and the overexpression of two distinct *kay* isoforms, we employed optical and scanning electron microscopy (SEM) to assess the resulting phenotypes. Our findings reveal that the overexpression and loss-of-function for *kayak* generates defects in imaginal structures. We observed a spectrum of phenotypic outcomes across different alleles. While some alleles exhibited pronounced defects, others displayed almost no consequences. Remarkably, all investigated alleles share a common trait—embryonic lethality. Notably, the insertion of a P-element into the lncRNA also resulted in embryonic lethality and displayed a phenotype strikingly similar to *kayak* mutant alleles. These results shed light on the multifaceted nature of the *kayak* locus and its impact on development.

1180S **Understanding the relationship between soluble histone supply and gene regulation in the early embryo** Reyhaneh Tirgar, Mohit Das, Jared T Nordman Vanderbilt

Histones are essential for chromatin packaging and histone supply must be tightly regulated as excess histones are toxic. To drive the rapid cell cycles of the early embryo, however, excess histones are maternally deposited. Therefore, soluble histones must be buffered by histone chaperones. We have identified the chaperone necessary to stabilize soluble H3-H4 pools in the Drosophila embryo to be NASP. We observed NASP localization in the nucleoplasm throughout embryogenesis. In the absence of NASP, H3 levels are depleted in the early nuclear cycles until cycle 14, where H3 levels are stabilized. Although *NASP* null mutants are viable in Drosophila, *NASP*is a maternal effect gene. Embryos laid by *NASP* mutant mothers have a reduced rate of hatching and show defects in early embryogenesis. Since NASP is a H3-H4 specific chaperone, we hypothesize that reduced H3 and H4 levels in the absence of NASP may lead to differential chromatin packaging in the early embryo and consequently aberrant transcription. To test our hypothesis, we have generated NASP mutants that do not bind H3 and are using both FISH and RNA-seq based approaches to measure transcription. This work will could provide direct evidence that soluble H3 in the early embryo modulates transcriptional activity.

1181S **Homeostatic control of intestinal stem cell renewal by two transcriptional regulators** Victoria Placentra[1], Shilpi Verghese[2], Ken Moberg[1] [1]Cell Biology, Emory University, [2]Emory University

As organisms age, the proliferative capacity of stem cells declines, which in turn impairs tissue renewal and can lead to organ dysfunction and disease. How local and systemic cues precisely regulate stem-cell based tissue renewal remains unclear, particularly in the rapidly cycling intestinal epithelium. A pair of transcriptional coactivator proteins that promote intestinal stem cell renewal – the Hippo pathway protein YAP1 and the nuclear receptor (NR) interactor NCOA3 – are each implicated in the progression of colorectal cancers and have orthologs in the fruit fly, *D. melanogaster*: the nuclear coactivator proteins Yorkie (Yki; YAP1 in humans) and Taiman (Tai; nuclear receptor coactivator-3, NCOA1-3 in humans). Prior studies have established that a physical interaction between Yki and Tai mediated via respective WW (tryptophan-tryptophan) domains and PPxY (proline-proline-x-tyrosine) motifs allows these factors to cooperatively induce target genes associated with regenerative growth of disc epithelia (e.g., *Ilp8*). As Tai and Yki each drive intestinal stem cell (ISC) proliferation in the

adult *Drosophila* midgut, we sought to test the hypothesis that Tai:Yki binding is required for this gut renewal function, and that this interaction allows the Hippo and NR pathways to cooperatively regulate gut homeostasis via a set of as yet undefined target genes in midgut cells, e.g., absorptive enterocytes (ECs) and ISCs. To address this question, we have generated a CRISPR mutant fly stock in which the PPxY motifs of endogenous Tai are converted to PPxA, blocking Tai's binding to Yki. Remarkably, these animals are alive, but show evidence of susceptibility to chemical agents that induce gut damage. We will present evidence of cellular and molecular defects that underlie this defect, including analysis of changes in chromatin state in wildtype and *tai*^{PPxA} following gut damage.

1182S **Roles for *CG5755* in the testis and nervous system, and possible interactions with *Ant2* and cardiolipin** Linden Patterson[1], Maggie Woodward[1], Amelia Roselli[1], Claire Olson[1], Karen G Hales[2] [1]Department of Biology, Davidson College, [2]Biology, Davidson College

Dramatic mitochondrial morphogenesis during spermatogenesis makes the *Drosophila* testis a useful context in which to explore mechanisms of mitochondrial dynamics. We previously found that *CG5755,* also known as *SLC25A46b*, is required for mitochondrial shaping during late spermatid elongation. Its human ortholog, *SLC25A46*, is associated with several human neurodegenerative conditions. The expression pattern of *CG5755* suggests testis specificity, but another group reported neurological phenotypes in a knockdown. Our *CG5755/deficiency* flies indeed exhibited hyperactivity in climbing assays, with speeds faster than their wild type*, CG5755/CG5755, or +/deficiency* counterparts. We introduced a wild type *CG5755* transgene to *CG5755/deficiency* flies to ask whether the hyperactivity phenotype could be attributed to *CG5755* loss. The *CG5755* spermatogenesis phenotype was rescued, but hyperactive climbing remained in *CG5755/deficiency; CG5755*$^{+}$, indicating the hyperactivity is not due solely to loss of functional *CG5755* and may instead stem from interactions with a neighboring gene. Conversely, *CG5755* homozygous females but not males showed a reduction in climbing ability. To investigate additional functional aspects of *CG5755*, we used RNA interference (RNAi) to explore connections between *CG5755* and two other genes, *Ant2* and *cardiolipin-synthase (CLS)*. The human ortholog of Ant2 (SLC254A4/5) has been shown by others to interact with SLC254A46. *Ant2* knockdown revealed its role in male fertility, sperm motility, and normal testes development. We are further investigating whether RNAi knockdown of *Ant2* affects the localization of *CG5755*-GFP. The *CG5755* human ortholog's structural similarity to another protein previously predicted a possible interaction with the mitochondrial membrane-specific lipid cardiolipin. We found that localization of CG5755-GFP was unaffected by depletion of cardiolipin via *CLS* knockdown. However, *CLS* knockdown impaired testis development and size to varying extents, suggesting that cardiolipin may be important for germ cell proliferation and maintenance. Overall, these results suggest roles for *CG5755*, *Ant2*, and *CLS* in spermatogenesis though functional connections are still unclear.

1183S **Epithelial cell fusion is required for wound healing following UV-A irradiation** Minqi Shen, Lydia W Boer, Vicki Losick Biology, Boston College

Polyploid cells are life's stress responders. The more than doubling of a cell's genome is often an adaption that enables cells, tissues, and organisms to survive in response to injury, including from sources of genotoxic stress. Cell cycle dependent (endoreplication) or independent (cell fusion) mechanisms lead to the generation of mononucleated and multinucleated, polyploid cells. While endoreplication has been shown to enable polyploid cells to survive in presence of DNA damage, it remains unknown whether cell fusion leading to the multinucleated ploidy state provides equal protection. Here we utilize the adult *Drosophila* epithelium which we found responds to stress, including UV-A irradiation via the generation of mono- and multinucleated, polyploid cells. Exposure to UV-A irradiation results in cell loss and a corresponding wound healing response characterized by the generation of polyploid cells by cell fusion (dBrainbow color mixing) and endoreplication (nuclear ploidy $\geq$4C). In the adult *Drosophila* epithelium, cell fusion is the prominent response to UVA irradiation and essential for tissue repair. Epithelial specific expression of a dominant negative Rac GTPase via Gal4/UAS system blocks cell fusion post UVA exposure and impairs epithelial survival resulting in apoptosis. Whereas blocking endoreplication via epithelial specific knockdown of E2F1 or CycE does not appear to delay epithelial wound healing as cells are still able to fuse and restore tissue integrity. In conclusion, we have found that multinucleation via cell fusion is another strategy to protect against genotoxic stress. Thus we are aiming to elucidate whether these distinct ploidy states enable cell survival via similar or distinct molecular mechanisms in response to life's insults.

1185S **Live imaging reveals stepwise signaling and cytoskeletal changes underlying de-differentiation in the *Drosophila* testis** Carlie Relyea, Kari F Lenhart Biology, Drexel University

Tissue homeostasis requires continual maintenance of adult stem cells over the lifetime of an organism. These stem cells produce progenitors that go on to specialize and populate the tissue in which they reside. Yet, in many cases, insult, injury, and lifelong stress cause significant depletion of the stem cell pool which, in turn, produce insufficient progenitors to maintain the health of the tissue. Thus, the mechanisms that tissues employ to retain a robust stem cell pool are vital for prevention

of tissue degeneration and atrophy. One key method to renew the stem cell population is de-differentiation; the process by which differentiating cells of a tissue physically move back to the specialized stem cell niche and adopt self-renewing capabilities. The *Drosophila* testis is an ideal model to study the cell biology of de-differentiation as a number of both genetic and environmental perturbations induce this process to replenish the germline stem cell pool. Under homeostatic conditions, differentiating germ cells are released from the niche and go on to complete four rounds of mitosis with incomplete cytokinesis, forming interconnected units termed cysts. Each cyst is tightly encased by two quiescent somatic cells which co-differentiate, facilitating spermatogenesis and tissue maintenance. Work from our lab and others suggests a three-step model by which de-differentiation is achieved: 1. Somatic cells lose contact with adjacent germ cells 2. Germ cell cysts complete cytokinesis to release individual cells 3. Migration of germ cells back to the niche. Preliminary studies utilizing genetic analyses and longitudinal live imaging of germ cells have revealed the signaling and cell biological changes that promote two of these events, cytokinesis completion and migration. First, we find that completion of cyst cytokinesis is preceded by loss of F-actin at typically stable germ cell intercellular bridges, allowing for germ cell abscission outside of the niche. Moreover, subsequent migration back to the niche is mediated by Jak/STAT signaling, which promotes germ cell polarization and leading-edge cytoskeletal rearrangement. Current work is focused on live imaging somatic cells during de-differentiation to fully elucidate the mechanisms governing each step of de-differentiation to maintain the germline stem cell pool.

1186S **The *Drosophila* testis compensates for catastrophic germ cell loss by altering stem cell cytokinesis** Christie Campbell, Tiffany Roach, Kari F Lenhart Biology, Drexel University

Tissue homeostasis requires a balance in production of self-renewing stem cells and differentiating daughters, or progenitors. The specialized microenvironment, or niche, in which stem cells reside provide regulatory signals to control this balance. Diminished niche signals and subsequent progenitor loss, or atrophy, is a hallmark of aging. However, the mechanisms by which stem cells and their progenitors respond to tissue atrophy remain elusive. Using the *Drosophila* male germline as a model system, we explored a genetic model that depletes progenitors to mimic tissue atrophy through ablation by expression of an apoptotic factor, Hid. During homeostasis, germline stem cells (GSCs) engage a delayed cytokinesis program, the timing of which is controlled by niche derived Jak/STAT signaling. Following contractile ring disassembly, GSCs pause cytokinesis progress through formation of a secondary F-actin ring. The lifetime of that ring is controlled by Jak/STAT signaling and determines the timing of cytokinesis completion and release of daughter cells from the niche. While it is known that GSCs can increase their mitotic rate to compensate for progenitor loss, this compensatory mechanism comes with a cost due to the long duration of GSC cytokinesis. Previous work from our lab has shown that fast cycling GSCs are significantly more likely to exhibit failed cytokinesis, and thus, mitotic rate may be deleterious as a compensatory mechanism. Here, we elucidate a novel mechanism used by the testis niche to compensate for tissue atrophy. Ablation of germ cells leads to compensatory feedback to the niche, inducing faster secondary actin ring disassembly and significantly shortening the cytokinetic pause in GSCs. This altered cytokinesis timing is likely achieved through increased Jak/STAT signaling in the niche, mediated by increased niche size. We are currently investigating the hypothesis that differentiating somatic cells move back to the niche and transdifferentiate to increase niche size. Together, this data supports a model whereby under catastrophic germ cell loss, a larger niche is formed, providing increased STAT signal to support a faster release of progenitor cells. Future work will investigate the mechanistic steps underlying this feedback and subsequent change in niche architecture to better understand how stem cell niches attempt to prevent tissue atrophy.

1187S **Misregulation of germ cell cytokinesis drives tumorigenesis in the *Drosophila* testis** Beth Kern, Kari F Lenhart Biology, Drexel University

Tissue homeostasis requires a tight balance between maintenance of the stem cell pool and production of specialized daughter cells. This balance is accomplished by restriction of the stem cell microenvironment, or niche, such that only stem cells receive self-renewal cues. Dysregulation in the stem cell niche and surrounding tissues can induce progenitors to revert in identity, express stem cell-specific markers, and initiate tumorigenesis. Yet how progenitors on the path to specialization initially respond to pro-oncogenic signals, and the degree to which they convert to true stemness, remains elusive. Under homeostasis, the *Drosophila* male germline is maintained by a group of mitotically dividing germline stem cells (GSCs). Using a modified, but complete, cytokinetic program, GSC daughters are released from the niche and spatially displaced from niche cues. Outside of the niche, differentiating germ cells execute a highly conserved incomplete cytokinetic program. Here, formation of stable intercellular bridges termed ring canals (RCs) creates germline cysts that share cytoplasm and differentiate as synchronous units. Homeostasis of this tightly controlled system is disrupted by expansion of niche Jak/STAT signaling, which promotes formation of stem-like germ cell tumors (GCTs). Yet, the temporal events underlying transformation, and degree to which GCTs execute GSC programming, is unknown. Here, I find that initial reception of the Jak/STAT ligand Unpaired (Upd) by germline cysts does *not* promote full re-acquisition of GSC cytokinesis. Instead, GCTs have disrupted incomplete cytokinesis, exhibiting dynamic instability of F-actin at normally stable RCs. Persistent depletion of F-actin permits inappropriate completion of cytokinesis through abscission. Moreover, just as in mammalian GCTs, we find that defects in adjacent somatic

cells are sufficient to induce GCTs characterized by RC F-actin instability. This indicates that integrity of incomplete cytokinesis may require co-differentiation of somatic support cells. Our preliminary work suggests that somatic-derived FGF is required for fidelity of incomplete cytokinesis, is attenuated at tumor onset, and that aberrant cytokinetic completion drives germline tumorigenesis.

1188S **Exploring the complex signaling between a transcription factor, *dve* and a morphogen involved in establishing eye vs head fate.** Soumya Bajpai[1], Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Organogenesis is the development of a highly organized tissue from just a cluster of few cells. Although it sounds quite simple, but it involves interaction of multiple factors and signaling pathways. Morphogens and axial patterning factors are two important players in organogenesis. As morphogens impart positional information to determine cell fate while axial patterning drives the transformation of those cells present as a monolayer tissue into a 3-dimentional organ by establishing Dorsal-Ventral (DV), Anterior-Posterior (AP) and Proximal-Distal (PD) axes, among which DV axis is the first to form during eye development. Any aberration in establishment of either these axes or the morphogen gradient fosters craniofacial birth defects. And to better understand the development of these defects it is important to investigate the complex signaling that is involved in establishment of a normal eye vs head fate. *defective proventriculus* (*dve*), previously identified as a dorsal selector gene, is a K-50 homeodomain containing transcription factor, a *Drosophila* ortholog of human SATB1. Dve plays a crucial role in eye development since it›s loss-of-function of results in dorsal eye enlargement while gain-of-function results in complete eye suppression. The Hedgehog (Hh) family of molecules are an important class of morphogens and render a crucial part in eye development as it regulates formation of the retina in *Drosophila* by orchestrating a differentiation wave that allows for the rapid and precise differentiation of the fly retina. We found out that these two individually important players in eye development do associate with each other and affect the eye vs head fate. We further want to explore the signaling that comes into action because of this association and understand the cause of the aberrant establishment of eye vs head fate, a common problem observed in craniofacial birth defects. Here, we present our results from modulating Hh signaling pathway in *dve* expression domain and how it affects eye development.

1189S **Real-time brain lineage modulation in pregastrulation neural primordium *in vivo*** Kelli D Fenelon, Priyanshi Borad, Theodora Koromila Biology, University of Texas at Arlington

Cell differentiation, which drives embryonic development, is initiated by pioneer transcription factors (PTFs) that prime cells to shift their external roles by transforming their transcriptional landscapes. Neural progenitor specification and differentiation occur early in development with detectable neuroblasts arising a mere hour post gastrulation in the *Drosophila* embryo. Pre-gastrulation, pioneer factors activate a multitude of genes simultaneously to produce antecedent cells for burgeoning common lineages of distinct germ layers or tissue primordia. The roles of early PTFs involved in brain development, such as Odd-paired (Opa)/Zinc finger in the cerebellum (ZIC3) and Ocelliless (Oc)/OTX1/2, are largely conserved across the animal kingdom. Despite ongoing efforts, the synergistic functionality of PTF spatiotemporal cycles and their rippling long-term effects are poorly characterized. We have previously demonstrated a pregastrulation spatiotemporal niche of co-regulation by these two factors spatially confined to the early embryonic region of the future brain and occupying distal elements proximal to a panoply of brain genes. We develop *in vivo*, optogenetic and real-time transcription observation tools to modulate expression of these genes pregastrulation to interrogate the primordial fate determining events which specify the earliest brain lineages. Specifically, we are employing and engineering LEXY and LANS optogenetic transgenic control tools on endogenous and exogenous Opa and Oc allowing us to modulate pre- and post-gastrulation expression of novel real-time MS2 transcriptional reporters for brain genes (e.g. *hb, Dll*) *in vivo* in real-time with spatiotemporally precision. This work is vital to understanding the cell fate determinations which beget the coordinated cellular diversity of mature animalia.

1190S **Exploring Myoblasts and Muscle Development: The Impact of MEF2 Overexpression** Elizabeth Trujillo[1], Richard Cripps[2] [1]Biology, San Diego State University, [2]San Diego State University

The Myocyte enhancer factor-2 (MEF2) transcription factor plays a vital role in orchestrating the muscle differentiation program. While MEF2 cannot effectively induce myogenesis in naïve cells, it can potently accelerate myogenesis in mesodermal cells. This includes Drosophila imaginal disc myoblasts, where triggering premature muscle gene expression in these adult muscle stem cells has become a paradigm for understanding regulation of the myogenic program. Here, we investigated the global consequences of MEF2 overexpression in the imaginal wing disc myoblasts, by combining RNA-sequencing with RT-qPCR and immunofluorescence. We observed the formation of sarcomere-like structures that contained both muscle and cytoplasmic myosin, and significant upregulation of muscle gene expression, especially genes essential for

myofibril formation and function. These transcripts were functional, since numerous myofibrillar proteins were detected using immunofluorescence. Interestingly, muscle genes whose expression is restricted to the adult stages were not activated in these adult myoblasts. These studies confirm a broad activation of the Drosophila myogenic program in response to MEF2 expression, and suggest that additional regulatory factors are required for promoting the adult muscle-specific program. Our findings contribute to understanding the regulatory mechanisms governing muscle development, and highlight the multifaceted role of MEF2 in orchestrating this intricate process.

1191S **Nemp, a conserved IDR containing nuclear transmembrane protein, is essential for oogenesis** Ruichen C Cao[1], Yonit Tsatskis[2], Helen McNeill[1] [1]Developmental Biology, Washington University in St. Louis, [2]Cell Biology, The Hospital for Sick Children

The nuclear envelope is a highly regulated and organized double membrane that separates the nucleus from the cytoplasm in all eukaryotes. It is embedded with numerous regulatory and transport proteins. Nuclear envelope membrane protein (*Nemp*) is a highly conserved multipass inner nuclear membrane protein. Though *Nemp* is ubiquitously expressed in all examined tissues across mouse and fly, it exhibits strongly tissue specific phenotypes: one of the most striking results of *Nemp* loss is sterility, conserved across flies, female mice, zebrafish, and worms. In addition, point mutations in *Nemp* have been associated with early onset of menopause in humans. In plant homologs, Nemp family proteins have been implicated in chromatin tethering. The reasons for the strong tissue specific phenotypes of Nemp loss are unknown. *Nemp* genetic null flies are infertile, with atrophied ovaries that are incapable of producing eggs. Preliminary data shows early germline specific knockdown of Nemp leads to almost total loss of early egg chambers, while late knockdown has no apparent effect, indicating that Nemp functions in early oocyte development. Remarkably, egg laying can be partially rescued by concurrent knockdown of the cell cycle checkpoint protein Chk2, implying that the shrunken ovaries from Nemp loss of function are related to DNA damage checkpoint. I will present data characterizing the effects of inducible Nemp knockdown in meiosis and oocyte determination with the Gal4/Gal80 system. In addition, I will determine whether oocyte loss can be mitigated by loss of meiotic DNA breaks, and look for markers of DNA damage in Nemp loss of function.

Recent work in the lab has shown that Nemp contains intrinsically disordered regions (IDR) in its nucleoplasmic tail and forms puncta in mouse oocytes. Live imaging in mouse oocytes reveals these puncta to be dynamic and sensitive to mechanical stress. My preliminary data indicates that Nemp also forms puncta in *Drosophila* salivary glands, muscle and ovary. However, it is still unclear if these puncta are phase separated bodies, what the mechanism of Nemp phase separation is, if there are other components involved in these condensates, and what the biological function of these condensates are. I use *Drosophila* to explore some of these questions.

1192S **Systematic rectification of planar orientation angle leads to interface behavior switching and replenishment during epithelial remodeling** Timothy E Vanderleest[1], Liam J Russell[1], Katie E Linvill[1], Miao E Hui[2], Xie E Yi[2], Dinah E Loerke[3], Todd E Blankenship[2] [1]Molecular and Cellular Biophysics, University of Denver, [2]Biological Sciences, University of Denver, [3]Physics and Astronomy, Cellular and Molecular Biophysics, University of Denver

Tissue elongation through morphogenetic epithelial remodeling is a fundamental process that is used across a variety of multicellular animals and occurs through dynamic cell behaviors. In the early Drosophila embryo, tissue elongation is driven by the modification of local topologies through oriented cell intercalation. Previous work has shown that a system of planar polarity produces net contractile behaviors at vertical cell-cell contacts (or interfaces), i.e. those that are aligned along the dorsal-ventral axis. These interfaces contract fully to produce higher-order vertices, which then resolve into new horizontal (anterior-posterior aligned) interfaces through so-called T1 transitions. Here, we show that contractile events produce a continuous “rectification” of cell interfaces, in which interfaces systematically and continuously rotate towards more vertical orientations. Intriguingly, as interfaces rotate in angle, their behaviors change, often transitioning from stable to contractile regimes. This observation demonstrates that the planar polarized identities of cell-cell interfaces are continuously re-interpreted in time depending on their orientation angle. These behavioral transitions are observable at the molecular level, with interfaces acquiring higher levels of Myosin II motor proteins as they become more vertical. Disruptions to the contractile molecular machinery (F-actin and Myosin II) cause reduced rates of rotation, consistent with this dynamic rectification mechanism being driven, at least in part, by interface contraction. Importantly, as a result of this rectification scheme, the available pool of contractile interfaces is continuously replenished as new interfaces acquire contractile identities through rotation. This means that over time cells in the germband epithelium acquire additional interfaces that are capable of undergoing T1 transitions, thus allowing cells to participate in multiple staggered rounds of intercalary events, and thereby producing higher net tissue extension than would be possible in a system with only a single round of contractile vertical interface specification.

1193S **Competing for survival: Taiman controls fitness status by a mechanism involving glypican-dependent diffusion and capture of the Wg morphogen** Colby K Schweibenz, Ken H Moberg Cell Biology, Emory University School of Medicine

Cell competition ensures that the fittest cells populate developing primordia but is also postulated to underlie the phenomenon of "field cancerization," in which cancer cells expressing 'super-competitor' genes eliminate slow growing neighbors and take over an epithelial tissue. Our prior work demonstrated that cells overexpressing the *Drosophila* protein Taiman (Tai; human NCOA3/AIB1), a transcriptional co-activator of both the Ecdysone receptor and the Hippo pathway effector Yorkie, kill wildtype neighbors within the larval wing epithelium in a manner dependent on production of the Toll ligand Spätzle (Byun et al., 2019). Here we use the reciprocal finding that cells with reduced Tai expression (Tai^{low}) are competitive 'losers' as the basis of a genetic screen for mutations that rescue elimination of 'loser' cells . This screen recovered 'hits' in pro-apoptotic genes (*hid*, *reaper*), confirming a dependance of the 'loser' phenotype on apoptosis, and also recovered the *Drosophila* Adenomatous polyposis coli (APC) tumor suppressor homologs, *Apc1* and *Apc2*, which are conserved inhibitors of the Wg (Wnt) pathway. Notably, *tai* loss reduces expression of the Wg target gene *nkd* in wing discs while *tai* overexpression has the opposite effect, indicating that Tai normally promotes expression of a Wg pathway component(s). Subsequent work determined that *tai* promotes expression of the GPI-linked glypican *dally-like* (*dlp*), which localizes to the apical ECM to facilitate Wg spread from source cells and acts as a Wg coreceptor. tai^{low} regions of wing discs are depleted of Dlp protein and block extracellular spread of Wg protein from source cells, leading to the hypothesis that Tai^{low} cells are at a competitive disadvantage with wildtype cells for 'capture' of extracellular Wg. Ongoing experiments examine Tai effects on *dlp* RNA transcription and Dlp protein trafficking, with the goal of defining how Tai control of the apical extracellular matrix modulates competitive status.

1194S **Investigation of the genes and gene regulatory networks activated during regeneration** Noah C. Fryling, Rebeccah L. Messcher, Keisha Roldán Báez Roldán Báez, Shireen T. Shah, Gloria Uduehi, Lydia Driggers, Josh Pazin, Nikhil Reddy, Melanie I. Worley Department of Biology, University of Virginia

For the field of regenerative biology, there remain many unanswered questions, including when an organism regenerates, to what extent is regeneration accomplished via activating the same molecular mechanisms required for normal development versus regeneration-specific processes? To start to address this question, we use the model organism Drosophila melanogaster, which provides a wide availability of powerful genetic tools to support dissection of gene function in vivo, including the ability to screen gene interactions and quantify them through regenerative growth assays. Specifically, we study regeneration in imaginal discs, the larval precursor to adult structures such as eyes and wings. In larval organisms, imaginal discs are capable of robust regeneration with characterized stages, including the formation of a localized zone of proliferation and enhanced plasticity, called a blastema. Through single-cell analysis and genetic approaches, we previously identified a Gene Regulatory Network (GRN) that is critical for sustaining the pro-regenerative state of the blastema. This GRN is driven by the stress-responsive JNK/AP-1 pathway and the transcription factor Ets21C, which together form a feedforward loop that induces and sustains the expression of several key downstream effector genes, including genes that encode for several secreted ligands such as the unpaired cytokines. Ets21C is required for effective regeneration, as in the absence of Ets21C regenerative growth is prematurely terminated. Single-cell transcriptomic data was also used to identify potential genes of interest, including those significantly up-regulated during the regeneration of the wing disc that are not widely expressed during normal development. We show a pair of genes, wun/wun2 to be expressed spatially and temporally in the imaginal disc during regeneration. Current work consists of the characterization of regenerative growth in the absence of these genes, as well as other candidate genes shown to have altered expression during regeneration. Additionally, since a number of these candidate genes are implicated in cellular movement, a process likely essential for the early initiation of regeneration, we are determining the role of these genes in cell movement via tissue-specific knock-out. Our future work aims to elucidate the manner in which these candidate genes impact mechanisms necessary for regeneration.

1195S **The long chain fatty-acyl CoA reductase *waterproof* is required for eye and head development** Ashleigh Ogg[1], Amber Reynolds[1], Nia Harris[1], Adriana Visbal[2], Mardelle Atkins[3] [1]Sam Houston State University, [2]University of Houston-Downtown, [3]Biology, Sam Houston State University

In *Drosophila melanogaster*, many external adult structures arise from epithelial sac-like tissues known as imaginal discs. One such disc, the eye-antennal disc, gives rise to the adult eye, antennae, and head epidermis. Our lab performed an *in-silico* screen that identified 96 potential candidate genes for eye and head development. From this list, the gene *waterproof (wat)* was selected for further analysis. *Wat* encodes a fatty acyl CoA reductase (FAR), which is responsible for reducing very long chain fatty acids. Defects in FAR1, the human ortholog, can cause cataracts, spastic paraparesis, or fatty acyl-CoA reductase 1 disorder. The specific FAR encoded by *wat* is known to be involved in lipid metabolism. Current research demonstrates that *wat* is crucial for a functional embryonic tracheal system, however it has not been characterized in the eye-antennal imaginal disc. Using a *wat::GFP* fusion protein, we observed a restricted expression pattern in the eye-antennal imaginal disc that included Bolwig's nerve and photoreceptor axons. Surprisingly, knockdown of *wat* results in headless progeny when using *ey-Gal4* or *c311-Gal4*. Subsequent experiments were performed using the UAS-GAL4 transgenic system and immunohistochemistry to assess the tissue-specific requirements for *wat* expression. These experiments revealed

requirements for *wat* in cells of both the peripodial epithelium (PE) and disc proper (DP) cells, each producing a distinct phenotype affecting disc growth and differentiation. We will present these results as well as ongoing work to assess the role of *wat* in Bolwig's nerve and if this is important for structural integrity of the PE.

1196S **Subcellular Mechanisms of Programmed Cell Death in *Drosophila* Ovarian Nurse Cells** Georgette-Vanelle Wandji, Kristen Harder, Kimberly McCall Boston University

Cell death and clearance of cellular debris is essential to the development and homeostasis maintenance of an organism. These phenomena in *Drosophila* oogenesis play critical roles in ensuring the proper development of the female reproductive system and the formation of functional eggs. In the *Drosophila* ovary, germline-derived nurse cells (NCs) undergo non-apoptotic programmed cell death as part of normal development. We have found that NC death is controlled largely non-autonomously by the surrounding somatic stretch follicle cells (FC), and that the phagocytic machinery is required for the final steps of NC death: acidification, nuclear membrane breakdown, and DNA fragmentation. Lysosomal machinery acting in the surrounding FCs non-autonomously promotes the acidification and DNA fragmentation of nurse cells. Through candidate gene screens and confocal microscopy, we determined that V-ATPases (proton pumps) are enriched and localized to the plasma membrane of the FCs as they surround the nurse cells. Using a pH-sensitive reporter, we found that NC nuclei were not engulfed in vesicles, but instead acidification was observed outside the FCs. We propose that the FCs do not engulf the NC nuclei, but instead the V-ATPases promote extracellular acidification and the FCs release cathepsins to eliminate the NCs. To gain a comprehensive view of the molecular network that controls NC death non-autonomously, we will further elucidate how the cells' endoplasmic reticulum and plasma membrane are eliminated. We find that the NC membrane begins to break down prior to acidification and we hypothesize that the NC plasma membrane undergoes trogocytosis by the neighboring FCs. By observing the interaction between the plasma membranes of FCs and NCs during late-stage oogenesis, NC death and clearance will be better understood. This work sheds light on mechanisms of phagocyte-dependent cell death.

1197S ***cupid*, a spontaneous mutation affecting proximodistal patterning in *Drosophila*** Cory J. Evans[1], Juliana Venegas[2], Zoe Wong[2] [1]Biology, Loyola Marymount University, [2]Loyola Marymount University

A spontaneous mutation was isolated that behaves recessively, is located on Chromosome 3, and causes the formation of small adult wings and short forelegs. We have named this mutation *cupid* (*cpd*) because the appearance of these flies is reminiscent of Renaissance-era depictions of Cupid, often cherubic deities with small wings and short limbs. Recombination mapping with dominant markers place *cpd* within the 46-53 cM interval. Morphometric analysis indicates that wings of *cpd* homozygotes are approximately 68% of the size of wild-type (Oregon R) wings. Comparison of L3 vein length from its origin at the hinge to the anterior cross vein (ACV) and then to the wing margin indicates that *cpd* predominantly affects proximal wing development. From hinge to ACV, *cpd* flies average 38% of the length in wild-type flies, whereas from ACV to the margin, *cpd* flies average 86% of the length in wild-type flies. Additionally, most *cpd* flies (85%) have an incomplete or missing ACV, and vein L5 fails to reach the wing margin 60% of the time. In terms of length, complete L5 veins in *cpd* flies average only 75% that of wild type, whereas incomplete *cpd* L5 veins average 54% of wild type. Regarding legs, the major phenotype of *cpd* flies is shortening of the tarsus (78% of wt), apparently caused by the partial or complete loss of two or more of the five tarsal segments present in wild-type flies. This loss includes the distal region of tarsal segment T1 and is most noticeable in males because sex combs, which develop in this region, are always missing or strongly reduced in *cpd* male flies. In *cpd* flies, the tarsus appears to be composed of three segments, with the proximal region of T1 fused with T4 and followed by a normal T5 segment containing the tarsal claw. During our analysis, we became aware that mutations in *rotund* (*rn*), encoding a zinc-finger transcriptional regulator known to control proximodistal patterning, exhibit *cpd*-like phenotypes. Furthermore, *rn* maps to 48 cM on the third chromosome, coincident with the *cpd* interval. We find that null alleles of *rn* fail to complement *cpd*, with *cpd*/*rn* heterozygotes reproducing the *cpd* phenotype. Additionally, we find that a duplication of the *rn* locus carried on the second chromosome is sufficient to rescue the *cpd* phenotype. Collectively, our results indicate that *cpd* is allelic with *rn* and is likely a genetic null. We are currently analyzing the molecular nature of the *cpd* mutation in the *rn* locus.

1198S **Stem cell mitophagy-dependent quiescence involves regulation of CycE levels and its localization at the mitochondria** Anne-Marie Pret[1,2,3], Miriam Gonzaga[2,3], Shelly Caisley[2,3], Sahiti Peddi[2,3], Cheryl Chan[2,3], Riya Keshri[2,3], Beatriz Estrada[2,4], Julie Mathieu[2,3], Hannele Ruohola-Baker[2,3] [1]Institute of Integrative Biology of the Cell Gif-sur-Yvette/ University of Versailles, [2]Biochemistry, University of Washington, [3]Institute for Stem Cell and Regenerative Medecine, University of Washington, [4]Institute of Stem Cell and Regenerative Medecine, University of Washington

Cells can undergo cell-cycle arrest, quiescence or diapause, at the G1/S transition to avoid apoptosis under stress conditions. This state is reversible when the stress is removed allowing cell-cycle reentry. Recently our lab has shown a common mechanism by which two different types of stem cells, *Drosophila* Germline Stem Cells (GSCs) and human induced Pluripotent Stem Cells (iPSCs), enter stress-induced quiescence. Surprisingly, unlike in other cell types, high levels of CycE are localized

at the outer mitochondrial membrane during the G1/S transition in the stem cells studied. Quiescence in these stem cells involves reduction of CycE, as well as that of mitochondria via mitophagy driven by the Ser/Thr kinase Pink and the ubiquitin ligase Parkin.

Presently, we are testing several mechanisms by which CycE regulation might occur during stem cell quiescence:

1. In order to determine whether direct CycE degradation is involved, we expressed CycE deleted of its PEST domain in *Drosophila* GSCs and found that irradiation stress permits the cells to remain in the cell cycle rather than entering quiescence unlike the control. This suggests that CycE must be degraded via the proteasome pathway involving ubiquitin E3 ligases. Therefore, we are testing whether CycE in *Drosophila* GSCs is ubiquitinylated and, if so, is Parkin responsible for CycE ubiquitinylation. We are presently using a similar strategy to test whether the degron domains in human CycE are necessary for quiescence of human iPSCs undergoing rapamycin-induced stress.

2. A candidate gene approach of genes involved in mitophagy, mitochondrial dynamics, and vesicular trafficking in *Drosophila* is being used to screen for those having a role in GSC quiescence. We will present localization of CycE in positive candidate gene loss and/or gain of function conditions. Positive candidate genes will also be knocked out in human iPSCs using the CRISPR/Cas9 methodology.

Determining the mechanism for CycE regulation in stem cells is critical to understand how diapause-like cancer cells can be made to re-enter the cell cycle and thus remain amenable for cancer chemotherapy.

1199S **The Gut-Brain Axis Unveiled: Aging and Tauopathy Models Reveal a Novel Impact of the Brain on Intestinal Stem Cell Differentiation** Tyler Jackson[1,2], Ye-Jin Park[2,3], Tzu-Chiao Lu[2,4], Christina Ko[5], Lindsey Ran[5], Yanyan Qi[2,4], Hongjie Li[2,4] [1]Cancer and Cell Biology Program, Baylor College of Medicine, [2]Huffington Center on Aging, Baylor College of Medicine, [3]Development, Disease Models, and Therapeutics Program, Baylor College of Medicine, [4]Department of Molecular and Human Genetics, Baylor College of Medicine, [5]Department of Neuroscience, Rice University

Complex eukaryotic organisms are composed of specialized organ systems that must participate in cross-tissue communication to ensure proper responses to environmental stimuli. The gut-brain axis plays a pivotal role in inter-tissue communication, crucial for organismal adaptation and homeostasis. This bidirectional interaction involves neuronal signals regulating digestion and hunger responses. While functional assays dominate gut-brain axis research, understanding molecular mechanisms impacting gut stem cell homeostasis remains limited. Leveraging aging and neurodegenerative disorders as models, we explore the gut-brain axis using Drosophila melanogaster, a model organism sharing incredible orthology with mammalian gut features. Preliminary data from the Aging Fly Cell Atlas revealed age-associated dysplasia in intestinal stem cells (ISCs), prompting investigation into the contribution of brain aging to this phenotype. Our lab generated single-cell sequencing data using a neuron specific Tauopathy model, providing a resource to decipher the effects that neuronal dysfunction may have on cell function in the gut. Data from this resource recapitulated the age-associated accumulation of gut ISCs and additionally revealed a unique subset of immature enterocytes (absorptive cells in the gut). These findings underscore the nervous system's direct influence on cell differentiation in the gut. Furthermore, I found there was dysregulation of well-characterized signaling pathways during ISC differentiation using cell-cell communication prediction software to produce additional support for this finding. In summary, our work uncovers novel consequences of brain dysfunction on gut homeostasis, prompting deeper exploration of cross-tissue communication.

1200S **Having a Heart: Biological and Mechanistic Roles for Slit Fragments in *Drosophila* Heart Development** Sahara C Harrington[1], Riley Kellermeyer[2], Ria Anand[1], Jonathan Taasan[1], Laureline Lequint[1], Thomas Kidd[1] [1]University of Nevada, Reno, [2]Stowers Institute

Congenital heart defects (CHDs) affect around 1% of live births in the United States, making it the most common birth defect. Interestingly, mutations in axon guidance genes lead to congenital heart defects in humans. *Drosophila melanogaster* is an excellent cardiac model for understanding the biological roles that conserved axon guidance genes, *slit*, *robo1*, *Dscam1*, and *tok,* play in heart development. While full-length Slit is required to repel the cardiac cells generating the lumen, little is known about the function of the Slit proteolytic fragments generated by the Tok protease. We have characterized the heart defects of *tok* mutants, and those of new CRISPR alleles of *slit.* The new alleles, Slit-Uncleavable (*slit-UC*) and Slit-N (*slit-N* only fragment), allow for fragment-specific genetic manipulations that provide insights into their roles in development.

Genetic rescue and molecular experiments show that the N-terminal Slit fragment, Slit-N, mediates cell-cell adhesion through heterodimerization with Dscam1 and Robo1 receptors. Remarkably, both *slit-N* and *slit-C* can rescue the migration and adhesion heart defects of *tok* null mutants. However, preliminary data shows that *slit-N* does not rescue *slit* null mutant

phenotypes. Preliminary data also indicates strong interactions with collagen-IV (Pericardin) and collagen-XV (Multiplexin) in the extracellular matrix may be leading to disorganization across heart-specific cells.

Our work establishes molecular and biological mechanisms for these genes and suggests they may function in the collective migration of the cells that generate heart valves in humans.

1201S **Antagonistic interactions between Dpp signaling and defective proventriculus determines eye versus head fate.** Anjali Sangeeth[1], Neha Gogia[1], Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Axial patterning, a crucial process during development, involves the delineation of three axes, such as Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD), is required for the transition of a monolayer organ primordium to a three-dimensional organ. Axial patterning involves the participation of morphogens, transcription factors, and regulatory genes. Any deviations in this process result in birth defects. We have identified a K-50 homeodomain-containing transcription factor, *defective proventriculus* (*dve*, an ortholog of SATB1), as a dorsal fate selector gene during eye development. Using genetic approaches, we found that Dve interacts with various signaling pathways during eye development to determine eye versus head fate. Among these, a highly conserved Decapentaplegic (Dpp)/Bone Morphogenetic Protein (BMP) signaling pathway, which forms a morphogen gradient in the developing *Drosophila* eye, acts antagonistically to *dve* to regulate retinal differentiation and thereby determines eye versus head fate. Here we present our studies on molecular genetic interactions between the members of Dpp signaling pathway and *dve* during eye development. Interestingly, SATB1, a mammalian homolog of *dve*, exhibits similar interaction with Dpp signaling pathway members suggesting that these interactions may be conserved.

1202S **Characterizing anterior somatic muscle specification in the *Drosophila* embryo** Lauren Kraker[1], Josue Merida[2], Dalia Fainberg[2], Kristelle Capistrano[1], Krista Dobi[2,3] [1]Natural Sciences, Baruch College, [2]Baruch College, [3]Molecular, Cellular and Developmental Biology, CUNY Graduate Center, City University of New York

Muscle wasting is a progressive decline in tissue mass and strength that occurs due to disuse, muscle diseases, cancer (cachexia) and aging (sarcopenia). Prevention and treatment of these disorders depends on our ability to generate different sizes and shapes of muscles, so that we: 1) understand why certain muscle types are affected and 2) can repair or generate new muscle tissues *in vitro* that match those affected muscles. Fruit flies make an ideal system to study muscle function because both fly and human systems feature multinucleated, striated muscles that form indirect attachments to tendons or direct attachments to skeletal structures. Over 20 identity gene transcription factors have been shown to regulate muscle properties in the *Drosophila* embryonic abdominal hemisegments. A few of these transcription factors have been examined in the T2 and T3 thoracic hemisegments, but almost no work has examined the development of the T1 muscle pattern that connects the somatic musculature to the mouthhooks used for feeding. One of the challenges of this muscle subset is that the embryonic head segment invaginates during head involution; the thoracic musculature correspondingly moves forward so that the most anterior external hemisegment becomes T1. We have begun our analysis of T1 using the live transgene *twist-actin-GFP* to follow the movements of the dorsal pharyngeal mesoderm and somatic mesoderm during head involution. These movies show that myoblast fusion continues during tissue rearrangement. A preliminary mutant analysis has shown that overexpression of *ap* or *muscle segment homeobox* using *twist-Gal4* leads to mispatterning of T1. In vertebrates, muscles can form indirect attachments to tendons or direct attachments to bone. We are particularly interested in the T1 subset of muscles to determine whether *Drosophila* larvae have these two types of attachments. We are using antibody staining and microscopy to determine whether muscle attachments to the internal cephalopharyngeal skeleton are structurally similar to muscle attachments to the external cuticle via tendons.

1203S **Ets21C-dependent gene regulatory network shared between regeneration and tumorigenesis** Keisha M Roldan Baez[1], Rebeccah L Messcher[2], Noah C Fryling[1], Shireen T Shah[1], Gloria Uduehi[1], Lydia Driggers[1], Josh Pazin[1], Nikhil Reddy[1], Melanie I Worley[1] [1]Department of Biology, University of Virginia, [2]University of Virginia

Regeneration is the process by which damaged or lost tissues are replaced with functionally equivalent tissues. Tumors share many characteristics with regenerating tissues, including increased cellular proliferation and loss of differentiation markers. We aim to better understand the molecular and cellular mechanisms that regulate growth during regeneration and tumorigenesis. To this end, we investigate regeneration and tumorigenesis in Drosophila imaginal discs. Through single-cell analysis and genetic approaches, we have previously identified a Gene Regulatory Network (GRN) that is critical for sustaining the pro-regenerative state of the blastema and demonstrated that this pro-regenerative GRN is co-opted to promote tumorous growth in a neoplastic-tumor model. This GRN is driven by the stress-responsive JNK/AP-1 pathway and the transcription factor Ets21C, which together form a feedforward loop that induces and sustains the expression of several key downstream

effector genes, including genes that encode for several secreted ligands such as the unpaired cytokines. We show that Ets21C is required for effective regeneration and that in the absence of Ets21C regenerative growth is prematurely terminated. The mammalian orthologs of Et21C, Ets-related gene (ERG) and friend leukemia integration 1 transcription factor (FLI1), are recognized proto-oncogenes that are associated with Ewing Sarcoma. Therefore, we were interested in characterizing the Ets21C-dependnet GRNs activated both during tumorigenesis and regeneration. To address this question, we compared single-cell data from an imaginal disc neoplastic tumor model to regenerating tissue. The tumor model is based on the loss of the apicobasal polarity regulator and forms disorganized and multilayered tumors. In these tumors, we identified subpopulations of cells that were blastema-like and demonstrated that Ets21C-/- mutants had reduced tumor growth, suggesting that these tumors hijack pro-regenerative pathways to promote their own cell proliferation and growth. We are currently investigating how overexpression of Ets21C impacts tissue growth and the function of several genes that are upregulated in subpopulations of cells in both regeneration and tumorigenesis. Our future work aims to elucidate the cellular and molecular mechanisms that regulate shared growth properties during regeneration and tumorigenesis.

1204S **Investigating the role of ecdysone signaling in dorsal closure using Halloween genes.** Jae Ho Lee[1], robert q ward[2] [1]Case Western Reserve University, [2]Biology, case western reserve university

20-hydroxyecdysone (20E) is a well-characterized steroid hormone required for major development changes in Drosophila. 20E surges before each larval molt, before pupariation, and during terminal differentiation of the adult structures. During molting and metamorphosis, 20E binds to its receptor to directly activate a group of early genes such as Broad-Complex (BR-C), E74, and E75, which activate late genes that are performing stage- and tissue-specific functions. There is also a less well-characterized pulse of 20E during mid-embryogenesis. Previous studies indicate a role for 20E signaling in germband retraction, head involution, dorsal closure, and cuticle secretion during embryogenesis. Here, we are focusing on the role of 20E signaling during dorsal closure in mid-embryogenesis. To gain a mechanistic understanding of the function of the 20E during dorsal closure we are characterizing phenotypes associated with loss of *disembodied* (*dib*) and *shroud* (*sro*), two “Halloween” genes that encode biosynthetic enzymes required for 20E synthesis. Our immunostaining images on *dib* and *sro* mutant embryos during dorsal closure show that JNK and Dpp signaling, which are initial signaling pathways of dorsal closure, appear normal. Likewise, the formation of the actomyosin cable on the leading edge is normal in mutant embryos. However, our time-lapse imaging of Ecad and Sqh marked *dib* and *sro* mutant embryos show poor formation of the canthus and zippering of the contralateral epidermal sheets. Also, we observe that mutant embryos show significantly slowed germ band retraction, abnormal shape and behavior of aminoserosa cells during dorsal closure, and reduced filopodia activity at the leading edge. Based on our observation, ecdysone seems to work in both the amnioserosa and epidermis for some aspects of actomyosin dynamics. To confirm this, we are also conducting tissue-specific expression of a dominant negative Ecdysone receptor (EcR-DN) to compare with the Halloween mutants. Preliminary data shows that ubiquitous expression (daughterless GAL4) of EcR-DN shows a strong dorsal closure phenotype. Surprisingly, expression in the amnioserosa via 332.3 GAL4 does not show dorsal closure defects by cuticle preparations. We plan to conduct Ecad and sqh live imaging in the ECR-DN lines to compare to our current results.

1205S **Lipid accumulation promotes loss of homeostasis in the male germline stem cell niche through differentiation** Rafael S Demarco Biology, University of Louisville

The capacity of stem cells to self-renew or differentiate has been attributed to distinct metabolic states. In the Drosophila testis, both germline stem cells (GSCs) and somatic cyst stem cells (CySCs) employ strategies to control lipid levels to promote stem cell maintenance. When lipid catabolism is impaired, neutral lipids accumulate in lipid droplets (LDs) and GSCs and CySCs exit the niche to differentiate, suggesting a role for lipid metabolism in the control of maintenance versus differentiation. We are currently investigating the role of LDs in stem cell maintenance, as well as the impact of lipid accumulation in the overall stem cell niche. Recent data suggests that lipid accumulation is detrimental for the maintenance of hub cells, a main component of the stem cell niche in the testis, by promoting hub-to-CySC conversion. Our findings highlight a critical role for lipid homeostasis in stem cell maintenance, providing a framework for investigating the impact of metabolic diseases on stem cell function and tissue homeostasis.

1206S **Molecular genetic dissection of a transcription factor Defective proventriculus in growth versus cell fate decisions** Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Defective proventriculus, a homeodomain containing transcription factor acts as a dorsal fate selector during *Drosophila* eye development. It is highly conserved across species and its human ortholog SATB1 is highly upregulated in cancer. Earlier,

we have shown that *dve* is crucial to establish dorsal fate which is required to promote growth and cell fate decisions. Furthermore, the *dve* expressing cells (~150-200) are responsible for expression of a morphogen *wingless (wg)/Wnt* in the developing eye. These *dve* expressing cells provide an inductive signal to determine eye vs head fate specification. Since *dve* is involved in growth regulation and cell fate decisions, we wanted to dissect these two independent functions during eye development. We employed structure-function analysis because *dve* has two isoforms and the Dve protein has multiple functional domains such as ULD domain, two Hox domains and a PPP4R2 domain. We hypothesized that different domains of Dve might be involved in regulating different functions. We present our results from studies on the role of these domain(s) on eye development and regulation of Wg expression in the developing eye. Here, we present results from individual functional domain(s) of Dve, combination of domain(s) and chimeras with SATB1. SATB1, human ortholog of *dve*, has similar domains and such studies can shed light on how SATB1 might function in diseases.

1207S **Dorsal fate selector gene *defective proventriculus (dve)* interacts with JNK pathway to determine dorsal eye fate** Sunanda Yogi[1], Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Organogenesis occurs in a highly coordinated manner by regulating several cellular processes. The c-Jun N-terminal kinase (JNK) signaling pathway is an evolutionary conserved pathway that provides crucial signals for cellular process like proliferation, growth, survival and cell death during development and disease. JNK pathway is also a master cell death regulatory pathway that is at the node of several cell death mechanisms. We have previously identified *defective proventriculus (dve)* (SATB1, mammalian ortholog of *dve*), a K50 homeodomain transcription factor as a novel dorsal fate selector gene. We have demonstrated that the *dve* expressing cells (~150-200) are a crucial signaling center that is required for the transcription of a negative regulator of eye development, *wingless (wg)* morphogen. Loss of these cells resulted in change of developmental fates and impacted organogenesis significantly. Therefore, we hypothesized that *dve* might interact with JNK signaling pathway members to regulate cell survival and growth during development. We have used classical Gal4-UAS system to perform gain-of-function and loss-of-function studies to misexpress JNK pathway members in the *dve* expression domain. Here, we present our results to demonstrate the interaction between the conserved JNK pathway and a dorsal patterning gene. This study might help to shed light on how a patterning gene might interact with other pathways to provide survival cues to regulate growth and development.

1208S **Glucose 6 phosphatase is required for male fertility and sperm-induced activation of embryonic development** Sheida Hedjazi[1], Tetsuya Miyamoto[2], Hubert Amrein[2] [1]Nutrition, Texas A&M Uinversity, [2]Cell Biology and Genetics, Texas A&M Uinversity

Maternal genes expressed during oogenesis play critical and well-defined roles in fertilization and early embryonic development. In contrast, much less is known about paternally expressed genes during spermatogenesis in fertilization and early embryogenesis. Here, we describe a novel role for Glucose-6-phosphatase (G6P) in male fertility and/or the initiation of early embryonic development. G6P is mainly known for its role in hydrolyzing glucose-6-phosphate to glucose during gluconeogenesis and glycogenolysis in the mammalian liver and kidney. Since trehalose, but not glucose, is the main circulating sugar in insects, and conversion of glucose-6-phosphate to trehalose requires the enzyme Trehalose phosphate synthase and not G6P, the latter has become dispensable in this process. Surprisingly, some insect genomes, including that of, have maintained a functional *G6P* gene. We previously showed that *Drosophila G6P* has a non-canonical role in a small subset of peptidergic neurons in the fly CNS, where it is required for neuropeptide signaling. We now report a second critical and non-canonical function for *G6P* in spermatogenesis. *G6P* mutant males produce phenotypically normal sperm and mate with the same frequency as wild-type males but produce virtually no progeny. Using the sperm marker *don juan-GFP,* we find that while *G6P* mutant sperm is deposited in female's spermathecae, and can penetrate eggs, its localization within the egg is disturbed compared to wild type sperm. Moreover, we find that *G6P* mutant sperm have an upregulated stress response, assessed by increased splicing of the unfolded protein response marker *Xbp1*. Taken together, we propose that G6P plays a pivotal role in fertilization and possibly the subsequent commencement of embryogenesis.

1209S **Novel role of miRNA-137 in Drosophila eye development** Radhika Padma[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

During organogenesis various players including genes non-coding RNAs like microRNA (miRNA) are involved in differentiation and development. miRNA are involved in post-transcriptional regulation of gene expression in a tissue-specific manner and

thereby fine tune the expression of their target gene(s). Therefore, miRNAs play a crucial role in regulation of the cell fate and differentiation during development. In a forward genetic screen, we identified a miRNA which exhibits reduced eye phenotype upon overexpression in the developing eye. Here, we present our characterization of the role of this new miRNA-137 (miR-137) in growth and development of an eye-antennal imaginal disc of Drosophila. Our results illustrate the novel role of miR-137 in eye development and growth. The results from our studies on molecular characterization of the role of this miR-137 during eye development will be presented.

1210S **Me31B function is required during adult muscle myogenesis in *Drosophila*** Harry Manning[1,2], Maria L Spletter[1] [1]Biological and Biomedical Systems, University of Missouri Kansas City, [2]University of Bath

RNA-binding proteins play important roles during myogenesis, both in directing alternative splicing to produce multiple isoforms of fiber-type specific proteins as well as in regulating the nuclear export, trafficking, stability, and translation dynamics within the muscle cell. This determines the balance in isoform expression of sarcomere proteins, enabling muscles to fine-tune their morphologies and contractile properties. Misregulation of RNA-binding protein function is observed in muscle aging and disease. Indirect flight muscles (IFMs) in *Drosophila melanogaster* are a powerful model to study RNA-binding protein function in muscle development. We identified a novel muscle-specific role for the DEAD-box RNA-helicase Me31B in adult myogenesis in an RNAi screen. Me31B has been previously reported to localized to P-bodies and mediate repression of maternal transcripts through translation repression and regulation of mRNA stability in the fly embryo. We show that me31B is expressed at a high level in muscle cells and localized at the nuclear membrane in distinct punctate structures in the indirect flight muscles (IFMs), consistent with a role in IFM development. Knockdown of *me31B* using Mef2-Gal4, which drives in all muscles, as well as with drivers Act88F-Gal4 and UH3-Gal4 that drive in early stages of IFM development result in pupal lethality. Late-stage IFM-specific knockdown driven by Fln-Gal4 did not produce a strong behavioral or cellular phenotype, suggesting Me31B function is important during early stages of IFM development. Structure-function analysis using CRISPR alleles targeting distinct Me31B domains further reveals Me31B function in muscle. Our data suggest a critical role for DDX6 homolog me31B in pupal muscle development, emphasizing the importance of RNA regulation during myogenesis.

1211S **Dose-dependent Effects of Lead Exposure on Development and Behavior in Drosophila melanogaster** Gabriella Zegarelli, Anna Arzeno, Sarah John, Dylan Koproski, Vanessa Yap, Rhea Datta Hamilton College

Lead (Pb) is a toxic metal that has adverse effects on neurological and reproductive development in various animal systems. In humans, the burden of lead exposure is not evenly distributed across the population, with low-income individuals and minority groups facing a disproportionately higher risk of exposure. In our research, we use Drosophila as a model to investigate the impact of dose-dependent exposure to Pb on various locomotive and reproductive behaviors, including sleep, hyperactivity, light sensitivity, fecundity, and egg-laying. We first assessed the effects of Pb exposure on larval and adult viability. Our findings reveal that both F0 and F1 control groups consistently produced more pupae and adult flies compared to all treatment groups. We then conducted assays on locomotion and activity. We found that Pb-exposure increased hyperactivity in flies during the masking-transition period from night to day and resulted in disruptions to circadian rhythms. Finally, we assessed the effects of Pb on fecundity and ovarian morphology. Notably, ovarioles in Pb-exposed experimental flies exhibited qualitative differences, with higher Pb concentrations causing increased fragility, compared to control and lower-dose experimental groups. However, fecundity increased at exposure to low concentrations of Pb and decreased at higher levels of exposure. Our data suggest that Pb exerts a dose-dependent influence on the development and behavior of Drosophila melanogaster. It remains unclear however, how Pb affects the genome. We are currently examining the potential mechanisms underlying Pb's impact on the behaviors we outlined above by looking directly at the effects of Pb on loci that regulate locomotion and oogenesis.

1212S **Different Bru1 isoforms play functionally distinct roles during IFM myogenesis in Drosophila** Maria L Spletter[1,2], Jenna DeCata[3], Aaron Morgan[3], Elena Nikonova[4], Tobias Straub[4] [1]Biological and Biomedical Systems, University of Missouri Kansas City, [2]Physiological Chemistry, Ludwig-Maximilians-University Munich, [3]University of Missouri Kansas City, [4]Ludwig-Maximilian-University Munich

CELF (Bruno) family members are important regulators of RNA processing in muscle. Muscles fine-tune their morphological characteristics and contractile abilities through regulation of gene expression and alternative splicing. The balance in splice-isoform expression of sarcomere genes varies between muscle-fiber types and is disrupted in muscle diseases such as myotonic dystrophy (DM1). Misregulation of CELF activity is thought to pathogenically underly contractile defects and muscle atrophy in DM1, highlighting the importance of RNA regulation for muscle homeostasis. CELF proteins are themselves alternatively spliced, but isoform-specific functions are currently unknown. We previously demonstrated that Bruno1 (Bru1), a CELF homolog in *D. melanogaster*, is required for indirect flight muscle (IFM) development. Bru1 is required early in muscle development for cytoskeletal rearrangements to support myogenesis and controls a later switch in fiber-type specific alternative splicing that enables sarcomere growth and maturation. Here we report that different Bru1 isoforms play

distinct roles during IFM myogenesis. We show using RT-PCR that the majority of Bru1 splice isoforms are expressed in IFM. Unexpectedly and in contrast to null alleles, an isoform-specific mutant affecting long *bru1* isoforms progressed normally through early stages of myogenesis but then underwent hypercontraction in adult IFM. This phenotype can be rescued by expression of longer Bru1 isoforms with Fln-Gal4 during later maturation stages, indicating that a novel short Bru1 isoform with only two RRM domains is sufficient during early myogenesis, but longer three RRM containing Bru1 isoforms are necessary during later myogenesis. We further demonstrate differences in overexpression phenotypes as well as subcellular localization of Bru1 isoforms Bru1-RA, Bru1-RB and Bru1-RD. Our data are consistent with isoform-specific functions for Bru1 and challenge models suggesting that all three RRM domains in CELF proteins are necessary for target binding. We propose that alternative splicing of CELF proteins is a conserved mechanism to fine-tune regulatory activity and developmental splice-isoform transitions.

1213S **The transcription factor Xrp1 regulates organ size in Minute (Ribosomal Protein heterozygous mutant) flies** Amit Kumar[1], Nicholas E Baker[2] [1]Genetics, Alber Einstein College of Medicine, [2]Genetics, Albert Einstein College of Medicine

A complex interplay of genetic, cellular, and environmental factors regulates organ size during the development of an organism. The genetic pathway that controls organ size during the development of an organism is well studied, but how organ size is regulated upon altered growth is not yet well studied. We are working on mutants of the ribosome protein-encoding genes in *Drosophila*, commonly known as *Minute* mutants. The $Rp^{+/-}$ (heterozygous mutant) animals are slow-growing but attain the size of wild-type flies after a developmental delay. We have previously identified a transcriptional pathway, regulated by the bZip AT hook protein Xrp1 controlling multiple aspects of the $Rp^{+/-}$ phenotype, particularly cell competition, the cellular fitness sensing mechanism that eliminates sporadic $Rp^{+/-}$ cells in mosaics. Xrp1 also controls growth rate, translation, and gene expression changes in $Rp^{+/-}$ cells. Here we examined the development of $Rp^{+/-}$ mutant wings. Our results show that $Rp^{+/-}$ mutant wings need Xrp1 to attain the normal size. We are currently investigating if Xrp1 regulates wing size in $Rp^{+/-}$ mutants through Yki, or through any of its transcriptional target genes, and will present a model of Xrp1 function in organ size regulation.

1214S **Shared and divergent roles for Bithorax-Complex miRNAs in regulating reproductive behavior across *Drosophila* species** Binglong Zhang, Daniel L Garaulet, Eric Lai Developmental Biology Program, MSKCC

We have long-standing interest in the biogenesis and function of ~22nt miRNAs encoded by the *Drosophila* Bithorax Complex (BX-C), which is most well-known for encoding homeodomain transcription factors that specify anterior-posterior segmental identity (Hox factors). Curiously, bidirectional transcription of the same hairpin locus generates miR-iab-4 and miR-iab-8, which both obey core regulatory principles for Hox genes, namely colinearity and posterior prevalence. In particular, both miRNAs directly repress more anteriorly-expressed/5›-located BX-C Hox genes *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*), as well as the Hox cofactor *Homothorax* (*Hth*). The miRNA knockout (*Δmir*) exhibits profoundly defective reproductive behaviors, and is completely sterile in both sexes. Recently, we used precision genetic engineering to demonstrate that specific miRNA binding sites in the Hth 3'UTR are required for female sexual receptivity. Now, we engineered additional miRNA resistant alleles of *Ubx* and *abd-A* to examine their contributions to reproductive behaviors. To address if BX-C miRNAs are required in other species, we generated the first *Drosophila* miRNA knockout outside of *D. melanogaster*, namely in *D. simulans*. These mutants phenocopy overall anatomical and behavioral defects, but also exhibit certain differences. We wish to know if these may potentially relate to divergent circuitry underlying reproductive behaviors between these closely related species (Ruta lab). Finally, we wish to elucidate the circuit and target basis of incomplete male copulatory behaviors in (*Δmir*), which accounts for male sterility. Altogether, there are shared and divergent roles for BX-C miRNAs that are manifest in the CNS, and are fully required for fertility in both sexes in multiple species. The paradigm of BX-C miRNA function is of general interest to regulatory biology, given the vast majority of miRNA knockouts have only subtle phenotypes.

1215S **Investigating the development of key somatic cells in the *Drosophila* ovary** Joanna Portillo[1], Mark Van Doren[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

Throughout the animal kingdom, male and female gonads develop differently in a process that is regulated by the Doublesex and mab-3 related (DMRT) family of transcription factors. Our lab studies how *Drosophila* Doublesex (Dsx) controls development of the somatic gonad of the ovary and testis. A great deal is known about testis development, while much less is known about development of the ovary. Therefore, to understand how Dsx controls sexual dimorphism in the somatic gonad, we first need to understand how the somatic stem cells and support cells of the ovary form and attain their distinct identities. Our current theory is that the somatic support cells, Escort Cells (ECs), and the somatic stem cells, Follicle Stem Cells (FSCs), develop from a set of somatic cells known as "intermingled cells" (ICs) in the larval ovary. Single-cell RNA sequencing from the Lehmann lab demonstrated that the ICs in the larval gonad have distinct anterior vs. posterior identities. Recently, our lab has characterized the development of the FSCs using the FSC marker, Castor. We found that a pool of FSC precursors form from posterior ICs during early pupal stages and the JAK/STAT pathway is required for cells to take on FSC precursor identity at this time. Based on these observations, this work aims to investigate the role of signaling pathways on ECs and FSCs differentiation

from ICs. We have begun examining how the JAK/STAT and Hedgehog (HH) pathways influence EC and FSC development. Through the UAS/GAL4 system, we have manipulated different factors that influence the level of autonomous signal the cells receive from these pathways. We then visualized the change in cell identity and characteristics with confocal imagining through immunofluorescence and in-situ hybridization. We started by inducing a gain of function phenotype of in both the JAK/STAT and HH pathways. Overexpression of both results in over proliferation of ICs in larval gonads. Manipulating HH signals to ICs changes the expression levels of posterior IC marker, *bond*, suggesting that HH affects the identity of ICs. While the loss of JAK/STAT results in ovaries with no FSCs, loss of HH produces ovaries with small amounts of total cells. The gain of function in both pathways results in Castor expressed anteriorly in the germarium and EC marker, PZ1444-lacZ, expressed more posteriorly. The confusion in somatic cell identity suggests that both pathways are involved in the development of these cells.

1216S **Elucidating the non-autonomous role of macrophages in tumor growth regulation in *Drosophila*** Eri Hirooka, keisuke Iksawa, Shizue Ohsawa Biological Science, Graduate school of Science, Nagoya University

Tumor progression is not only driven by sequential acquisition of genetic mutations, but also regulated by interactions between oncogenic cells and surrounding normal cells in the tumor microenvironment. Growing evidence indicate that macrophages exert dual functions on tumorigenesis by both enhancing anti-tumor responses and antagonizing the anti-tumor immune cells. However, the underlying mechanisms remain elusive.

Using a Drosophila model system, We genetically analyzed the mechanisms of tumor progression through cell-cell communications. We found that malignant tumors, induced by Ras activation with cell polarity defects (Ras^{V12}/$scrib^{-/-}$) markedly recruit *Drosophila* macrophages, termed hemocytes, in the imaginal epithelia. Genetic ablation of hemocytes significantly reduced the size of Ras^{V12}/$scrib^{-/-}$ tumors, suggesting that hemocytes promote overgrowth of Ras^{V12}/$scrib^{-/-}$ cells. To explore the role of hemocytes in promoting Ras^{V12}/$scrib^{-/-}$ tumor growth, we genetically inhibited various signal pathways by expressing *RNAi* in hemocytes. The mechanism by which hemocytes non-autonomously regulate tumor growth will be presented.

1217S **Direct and indirect regulation of EGFR target genes by opposing gradients of BMP and JAK/STAT signaling patterns the follicular epithelium of the Drosophila ovary.** Laura Nilson[1], Kelvin Ip[2], Scott De Vito[3], Baptiste Rafanel[4], Kaitlin Mac Donald[2] [1]McGill Univ, [2]Department of Biology, McGill University, [3]Department of Pharmacology, McGill University, [4]Institute of Molecular Biotechnology, Austrian Academy of Sciences

A relatively small number of signaling pathways drive a wide range of developmental decisions involving distinct target genes. This versatility points to a role for cellular context in generating diversity in signaling outcomes, but how targets are chosen is not well understood.

Context-dependent signaling outcomes occur during patterning of the Drosophila follicular epithelium, where activation of epidermal growth factor receptor (EGFR) signaling by its localized ligand Gurken (Grk) induces distinct cell fates depending on its location. Posterior follicle cells respond to Grk by expressing the T-box transcription factors Midline (Mid) and H15, while anterior cells respond by expressing the homeodomain transcription factor Mirror (Mirr). The choice between these alternative EGFR targets depends on input from additional localized ligands. At the posterior, activation of JAK/STAT signaling by the ligand Upd is required for Mid/H15 expression, while at the anterior activation of BMP signaling by the ligand Dpp is required for Mirr expression. This choice is reinforced by mutual repression between Midline/H15 and Mirror, and through the ability of both Dpp and Upd to repress their “opposing” target (i.e. *mid/H15* and *mirr*, respectively).

The integration of these multiple localized positional cues suggests that crosstalk between these signaling pathways generates mutually exclusive EGFR signaling outcomes, but at what level this occurs remains unclear. To approach this question, we identified genomic regions from the *mid* and *mirr* loci that, in a transgenic reporter, recapitulate the expression patterns and regulation of endogenous *mid* and *mirr* genes. We have shown that these regions contain STAT92E and MAD binding sites that are required for Upd- and Dpp-mediated repression of *mirr* and *mid* reporters, respectively. Further mapping of these genomic sequences suggests that these repressive elements silence the ability of nearby sequences to positively regulate reporter expression. Interestingly, activation of *mid* reporter expression does not require putative STAT92E binding sites, suggesting that the positive regulation of *mid* expression by Upd is indirect. Indeed, we find that Upd positively regulates *mid* by increasing levels of the EGFR effector Pointed (Pnt), suggesting crosstalk in which Upd ensures levels of Pnt adequate to allow EGFR to induce mid expression. Dpp and Upd therefore influence EGFR target gene expression through both direct and indirect mechanisms.

1218S **A conserved Immunoglobulin cell adhesion junction module maintains epithelial integrity** Tara M Finegan[1], Dan T Bergstralh[2] [1]Biology, University of Missouri, [2]Division of Biological Sciences, University of Missouri

Epithelial tissues are sheets of cells that line animal body compartments to protect organs mechanically and chemically. Epithelial cells often move apically during mitosis with respect to the tissue layer and daughter cells can be born misplaced from the plane of the tissue. We found that misplaced cells can reincorporate back into the parental tissue layer through a process we call cell reintegration. Reintegration appears to be a general property of epithelia to protect their integrity and is likely to be a protective mechanism against carcinoma. In fruit flies, reintegration relies on a suite of evolutionary-conserved Immunoglobulin cell adhesion (IgCAM) proteins called Neuroglian (Nrg), Fasciclin II (Fas2), and Fasciclin III (Fas3). These proteins are well-studied for their roles in axon fasciculation, and we have previously demonstrated mechanistic overlap between this process and reintegration. Nrg, Fas2 and Fas3 are also identified at neuromuscular junctions (NMJs), synapses that appear subsequent to fasciculation, and in epithelia at septate (occluding) junctions, which do not develop in the follicular epithelium until after proliferation has ceased. These observations suggest both a mechanistic and an evolutionary relationship between neural and epithelial tissue development. We are currently exploring this possibility using structure-function analysis, genetic rescue experiments, quantitative analysis and advanced imaging techniques.

1219S **Investigating how niche shape coordinates stem cell behavior** Gabriela Vida, Elizabeth Botto, Stephen DiNardo University of Pennsylvania

Stem cells are important for repairing and regenerating our tissues, and often reside in a niche that controls their behavior. The testis niche has been a paradigm for niche-stem cell interactions. Recently, our lab has focused on the construction of this niche. Examining initial assembly of the niche in embryonic gonads has revealed both how extra-gonadal signals drive niche assembly (Anllo and DiNardo, 2022), and begun illuminating the cell mechanics involved in first forming a compact, functional niche (Warder et al. 2023). Here we use the adult testis niche to address the cell biological features that maintain niche structure and function during its steady-state operation. The niche resides at the testis tip and is comprised of a group of quiescent cells that send renewal signals to the neighboring stem cells. These quiescent cells are organized spherically, and are radially surrounded by two stem cell populations, the germline stem cells (GSCs) and the cyst stem cells. Additionally, this niche helps orient GSC divisions by centrosome anchoring at the niche to produce a renewing GSC and a differentiating daughter cell to achieve stem cell balance. Proper centrosome orientation is ensured by the centrosome orientation checkpoint (COC), which stalls GSCs in G2 until the centrosomes are properly oriented for mitosis. Our preliminary evidence suggests that acto-myosin contractility (AMC) is important for the maintenance of the spherical nature of the stem cell niche. This abolishment of this spherical structure reduces the effectivity of niche signaling per GSC, and instead allows for more germ cells to respond to signaling. This is accomplished by differentiating daughter cells making contact with the niche, potentially disrupting the differentiation program. Finally, this loss of niche structure causes the GSCs to misorient their centrosomes, bypass the COC, and execute misoriented divisions. Ultimately, this work shows a novel way in which niche structure ensures a proper balance of stem cells and is crucial for their behavior.

1220S **FMRP is a target of TDP-43 and mitigates morphological and functional phenotypes in striated muscle** Brijesh S Chauhan[1], Samantha Macklin[2], Daniela Zarnescu[3] [1]Cellular and Molecular Physiology, Penn State College of Medicine, [2]Cellular and Molecular Physiology, Penn State college of Medicine, [3]COM, Penn State COM

Amyotrophic Lateral Sclerosis (ALS) and Fronto-Temporal Dementia (FTD) comprise a spectrum of neurodegenerative disorders for which there is no cure. A major hallmark of pathology in ALS/FTD is the presence of cytoplasmic TDP-43 inclusions, which are linked to 97% of ALS and 45% of FTD cases. TDP-43 is a DNA/RNA binding protein with multiple roles in RNA processing including mRNA localization and translation. In axons, TDP-43 was shown to regulate the transport and translation of specific mRNAs while puromycin incorporation experiments show that cytoplasmic TDP-43 inhibits translation globally. Using ribosomal tagging approaches, we have recently shown that TDP-43 affects the association of mRNAs with ribosomes causing alterations in translation itself, cellular energetics, and synaptic function among other pathways. These findings underscore TDP-43's involvement in translation, however, its targets, and their impact on the homeostasis of neuromuscular synapses remain a poorly understood aspect of TDP-43 biology and its relationship to neurodegeneration in ALS/FTD. Here we explored the muscle side of the neuromuscular junction (NMJ) where local translation is known to occur. We examined TDP-43's impact on new protein synthesis using Non-Canonical Amino-acid tagging (NCAT) *in vivo,* in the context of Drosophila models of TDP-43 proteinopathy. These experiments show that overexpression (OE) of wild-type TDP-43 in muscles reduces new protein synthesis globally, with mutant TDP-43 showing a more pronounced reduction in translation. Using biotinylation and mass spectrometry in conjunction with NCAT (BONCAT) we identified several candidate targets of TDP-43 in muscles including the Fragile X mental retardation protein (dFMRP). Immunofluorescence experiments show that FMRP levels are significantly reduced in muscles overexpressing TDP-43 in flies. Interestingly, deletion of endogenous TDP-43 (TBPH) causes reduced FMRP expression consistent with TDP-43 OE mimicking a loss of function phenotype in muscles. Notably, FXR1 the muscle specific ortholog of dFMRP in humans exhibits a significant reduction in ALS patient muscles compared to control, consistent with our findings on dFMRP in flies. Lastly, overexpression of FMRP in muscles mitigates TDP-43 associated phenotypes including

locomotor deficits, reduced survival and sarcomere abnormalities, and suggest that restoring FMRP expression in muscles provides a potential therapeutic strategy for TDP-43 proteinopathies.

1221S **The Drosophila DPP/BMP-4 gradient formation in the embryo is dependent on cell constriction mediated by Frazzled and E-Cadherin** Aravindan Krishnan, Runze Ni, Hainvi Gullapalli, Rui Sousa-Neves, Claudia Mizutani Case Western Reserve University

A long-standing problem in developmental biology is how cells acquire positional information from morphogen gradients while being displaced in space. We showed that the DPP/BMP gradient attracts cells and precisely control their movements in the blastoderm through an interaction between the epithelial polarity genes *frazzled* (*fra*) and *guk-holder* (*gukh*), and the cell junction protein E-cadherin (ECAD). These stereotyped movements fine-tune the shape of the BMP gradient and establish precise cell fates (Xue et al., 2023, PLoS Biol). These results show that cell movements enable precision, rather than hinder cell fate specification. These findings open exciting new perspectives. For example, we showed that the formation of a peak level of BMP signaling in the dorsal midline, visualized by the intense dorsal stripe of pMAD, involves the contraction of dorsal cells, but the molecular mechanisms involving FRA, GUKH and E-CAD in this process remain unclear. Another question is how FRA maintains high levels of ECAD in the dorsal region of the embryo in an environment with high BMP signaling, which like in cancer, normally disrupts cell junctions and cause cell invasion. To answer the first question, we are testing whether ECAD form complexes with BMP receptors that become spatially denser as cells contract, leading to higher BMP activation. Using super-resolution microscopy, we found that ECAD and the BMP receptor TKV indeed co-localize in the dorsal region of the embryo. Cells within the pMAD stripe have less numbers of ECAD-TKV complexes compared to adjacent cells. This is a similar pattern to the developing wing disk, where TKV levels are lower within peak regions of the gradient, and higher in regions of low BMP. We are testing two possibilities to explain how a lower number of ECAD-TKV complexes can transduce higher levels of BMP signaling within the pMAD stripe region. First, we are determining whether ECAD-TKV complexes form high density clusters at the dorsal midline where the cells are more constricted and more disperse clusters in lateral regions. We are also testing whether ECAD-TKV complexes are more internalized due to TKV activation within the pMAD stripe and therefore located within the cell rather than the membrane. Finally, to answer how FRA maintains high ECAD levels, we hypothesize that FRA/ECAD are targets of Presinilin and FRA protects ECAD from excessive cleavage by competition. We show that Presinilin loss-of-function embryos have the expected increase in BMP activity compared to controls. We are testing if this effect agrees with less cleavage of FRA and/or ECAD, and with higher cell constriction. In sum, our results bring a previously unexplored regulatory component in the DPP signaling cascade and gradient regulation.

1222S **Characterization of Juvenile hormone esterase and Juvenile hormone epoxide hydrolase mutants** Rebecca Spokony[1], Harry Siegel[2], Said C Yashaev[3], Creehan Healy[2], Lacy Barton[2] [1]Natural Sciences, Baruch College, [2]University of Texas at San Antonio, [3]Baruch College, CUNY

Juvenile hormone (JH) is one of the most important regulators in insect development. It is synthesized through a series of enzymatic reactions and degraded by three classes of enzymes. Two of these are JH esterase (Jhe) and JH epoxide hydrolase (Jheh). Jhe and Jhe duplicate (JheDup) are next to each other on the right arm of the 2nd chromosome. Jheh is triplicated in tandem on the same chromosome. We developed new CRISPR mutants that are null for all copies of each class of gene, *Jhe, JheDup* and *jheh1,2,3*. *Jhe, JheDup* grow larger, while *jheh1,2,3* are smaller and pupariate 3 days after wildtype larvae. Here we examine if these phenotypes can be genetically rescued; if these phenotypes are epistatic to JH synthesis and if knock-down of a single enzyme is sufficient to cause these phenotypes. We used P[acman] BAC clones with approximately 80 kb of genomic DNA covering the deleted regions, inserted back into a complementary chromosome (3rd). Both size and timing phenotypes were rescued. JH acid methyltransferase (Jhamt) is the rate limiting enzyme converting inactive JH precursors into bioactive molecules. To test if a lower concentration of JH could reduce or eliminate the degredation enzyme phenotypes, we generated *Jhe, JheDup; Jhamt* and *jheh1,2,3; Jhamt* stocks. Elimination of Jhamt eliminated overgrowth of Jhe, JheDup. Preliminary results suggest an effect on *jheh1,2,3* timing defects. Lastly, we are currently testing a variety of RNAi lines ubiquitously driven by da-Gal4. Knock-down of Jhe alone lead to an increase in pupal size. Surprisingly, we preliminarily found that knocking down just one of any of the Jheh paralogs leads to an increase, rather than a decrease in size. We are currently checking efficiency of the knock-down with qPCR, and any compensatory changes in Jheh expression. We plan to examine which tissues are responsible for these phenotypes next.

1223S **Grainyhead regulates wound-induced polyploidization in Drosophila** Lydia M Bischoff, Erin C Bailey, Vicki P Losick Biology, Boston College

In Drosophila, adult epithelial wounds heal by cell growth instead of cell division. Cells grow orders of magnitude through polyploidization, which in fruit flies arises by both cell fusion and endoreplication at a puncture wound site. Previous studies have found that wound-induced polyploidization is required for epithelial wound healing as it permits wound closure in the

presence of genotoxic stress, restores tissue mass, and maintains tissue tension post-injury. What remains poorly understood are the genes that regulate polyploid growth in response to injury. The transcription factor, Grainyhead (Grh), is known to play a conserved role in regulating epithelial differentiation and wound healing by controlling the expression of cell cycle and extracellular matrix genes. Therefore, we investigated whether Grh contributes to the regulation of wound-induced polyploidization. Indeed, we have found that Grh regulates entry and the extent of endoreplication. Grh overexpression appears to be sufficient to induce endoreplication even without injury in epithelial cells. Previous research in our lab identified another transcription factor, Yorkie (Yki) which also regulates the extent of epithelial endoreplication. Thus, we are investigating whether Grh and Yki genetically interact to regulate endoreplication during wound healing.

1224S **Complementary Volume Electron Microscopy-based approaches reveal ultrastructural changes in germline intercellular bridges** Irina Kolotuev[1], Abigayle Elsbury[2], Stephanie Pellegrino[2], Lindsay Lewellyn[2] [1]University of Lausanne, [2]Butler University

Intercellular bridges are essential for the development of eggs and sperm in many organisms; however, despite their importance, there is still much to be learned about how their structure changes through development. The egg chamber is an excellent model system to study intercellular bridges. Within the egg chamber, the germ cells are connected to each other by relatively large intercellular bridges, or ring canals, which expand ~20-fold in diameter through oogenesis. Many proteins have been identified that localize to the germline ring canals and regulate their formation, growth, or stability, but a more extensive analysis of ultrastructural changes is lacking. Electron microscopy is a valuable tool that can be utilized to monitor structures through development or following genetic or other manipulations. Here, we provide an extensive ultrastructural study of the developing egg chamber using a combination of Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and Array Tomography Scanning Electron Microscopy (AT-SEM). We have rendered multiple large data sets collected using FIB-SEM to generate 3D models of multiple early-stage germ cell clusters. This analysis revealed interesting structural differences in the germline ring canals through development as well as within a single cluster; however, we quickly approached the functional upper limit (spatially and computationally) of FIB-SEM. Therefore, to monitor ring canal structure at later stages of development, we turned to AT-SEM. This approach allowed us to easily screen through the array of sample sections to identify the position of the ring canals within a much larger sample volume. Because we collect serial sections, we are still able to generate 3D reconstructions using this approach. Therefore, the combination of FIB-SEM and AT-SEM allowed us to monitor ring canal structure in 3D throughout oogenesis. Although the focus of this work has been on the germline ring canals, because electron microscopy reveals the entire morphome of the sample, we envision that this dataset could be utilized by other researchers in the field to answer a variety of questions.

1225S **Yorkie Activation Restores Epithelial Integrity in Neoplastic Tumors in Drosophila Wing Discs** Vanessa Ghorayeb, Gayaanan Jeyanaathan, Meggie Cao, Parama Talukder, Ulrich Tepass University of Toronto

Our recent work has established a link between mitosis and tumor progression in epithelial tissues. Using the Drosophila wing disc epithelium as a model, we have found that enhancing cell division by overactivation of E2F1, a cell cycle regulator, not only causes an increase in tissue size, but also exerts morphogenetic stress on the epithelium resulting in permanent loss of epithelial integrity, leading to neoplasia. This is seen in different conditions as well such as in neoplastic tumor mutants like *scribbled (scrib)* or with *crumbs (crb)* overexpression. We have also shown that decreasing cell proliferation in classic neoplastic models such as *scrib* knockdown rescues neoplasia and restores the epithelium, further implicating enhanced cell proliferation as the driving force of not just tumor growth but also neoplastic tumor progression. In contrast, accelerated cell proliferation mediated by Yorkie (Yki) activation does not result in loss of epithelial integrity, causing hyperplasia rather than neoplasia. Yki is a downstream effector of the Hippo pathway and promotes tissue growth via transcription of pro-proliferative and anti-apoptotic genes such as CyclinE and Diap1, respectively. Dysregulation of the Hippo pathway and increased activity of Yki or its mammalian homologs YAP/TAZ have been implicated in numerous epithelial cancers. In the Drosophila wing disc, overexpression of Yki (Yki-WT), or its constitutively active form (Yki-3A) results in dramatic hyperplastic tissue overgrowth and significant epithelial folding.

To explain this discrepancy between neoplastic tumors and Yki-mediated hyperplastic tumors, both driven by excessive cell proliferation, we hypothesize that Yki has target genes that can stabilize and restore epithelial polarity, counteracting proliferation-induces morphogenetic stress. To test this hypothesis, we have expressed Yki-WT and Yki-3A in various neoplastic backgrounds and examined both epithelial integrity and levels of JNK signaling, another signature of neoplasia. Preliminary observations indicate that Yki activation can strongly suppress neoplastic development and rescue epithelial polarity in neoplastic tumor mutants. A significant reduction of JNK signaling is also observed. These findings suggest that Yki plays a dual role, enhancing proliferation and protecting epithelial integrity at the same time.

1226S **Characterization of muscle survival induced by nutrient modulation after traumatic injury in *Drosophila*** Yutian Li California Institute of Technology

Muscles can repair daily wear-and-tears and minor strains. However, in more serious injuries, for reasons not well understood, injured muscles degenerate. While large muscle loss has been modeled in mammalian models, invertebrate models have a track record in helping discoveries of basic mechanisms. Toward developing an invertebrate model for studying large muscle injury, in this work, we assessed in adult *Drosophila* how limb muscle responds to dramatic injury, i.e., amputation, and identify a condition that can modify that response. As expected, muscle in the amputated limb rapidly degenerates within a week after injury. Muscle degeneration, we further find, can be significantly inhibited by administering the growth hormone insulin and the amino acids leucine and glutamine to the fly amputees. Consistent with the muscle survival, reporter of Notch signaling, known to be essential in muscle regeneration, is indeed activated in treated limbs. Finally, transcriptomic analysis suggests that the day-scale muscle survival is underlined by modulation in gene regulation occurring within hours, including modulation in genes involved in ecdysone signaling, immune response, and reproductive behavior. This work provides a starting point for investigating in a genetically tractable system the mechanisms that normally limit muscle survival upon severe injuries, and the molecular strategies that can overcome those limitations.

1227S **Mechanistic basis of germ-layer emergence by Polycomb Repressive Complex 1 in stem-cell derived embryonic organoids.** Shreyasi Mukherjee[1,2], Robert E. Kingston[1,2] [1]Department of Molecular Biology, Massachusetts General Hospital, [2]Department of Genetics, Harvard Medical School

Polycomb repressive complexes [PRC] are critical regulators of lineage fidelity across embryonic development, organogenesis, and tissue homeostasis; and their dysregulation is frequently associated with developmental disorders and cancer. PRC1 members have well-defined functions in maintaining pluripotency, and yet, how they spatiotemporally regulate the lineage commitment potential of stem cells remains unknown. During gastrulation, PRC1 members are highly expressed in the epiblast and primitive streak before exhibiting distinct expression patterns in the three germ-layer derivatives, indicating functional roles in regulating key decision points during lineage segregation. Here, we use embryonic stem-cell derived 3D organoid models of mouse gastrulation [gastruloids] to define the molecular consequences of PRC1 loss during germ layer specification. Our data indicates that loss of the PRC1 catalytic component RING1B inhibits axial elongation and significantly disrupts the progression of epithelial to mesenchymal transition upon gastrulation onset. In addition, RING1B-null gastruloids exhibit delayed WNT signaling responses and fail to spatially segregate the expression domains of mesendodermal markers. In contrast, loss of CBX and PHC family members lead to delayed gastrulation defects and ectodermal expansion. These results identify multiple complex and subunit-specific contributions of distinct PRC1 members in initiating gastrulation and specifying cellular identities in distinct germ-layers. Collectively, these data have the potential to shed light on how Polycomb complexes integrate spatiotemporal cues to direct critical lineage segregation decisions during early embryogenesis.

1228S **Non-ciliary roles for the cilia-motility associated gene *Cfap298*(*Kurly*) in zebrafish and mouse** Marvin Cortez, Rebecca Burdine, Danelle Devenport Princeton University

Cfap298 (*Kurly*) is important for regulating cilia/flagellar motility and is conserved across ciliates. Disrupting *Cfap298* function leads to ciliopathy-related disorders in both humans and zebrafish. Additionally, *Cfap298* has also been shown to be important for motile cilia polarity in some tissues in *Xenopus* and zebrafish through the Planar Cell Polarity (PCP) mechanism. To investigate the role of CFAP298 in mouse we generated two alleles. The $Cfap298^{delta3aa}$ affects cilia motility and left-right patterning as expected. Surprisingly, our $Cfap298^{null}$ mutant results in early embryonic lethality between E4.5-E5.5 perimplantation stages due to a failure to maintain epiblast cells. This suggests that *Cfap298* plays important roles in development prior to the emergence of motile cilia. *Cfap298* mutants are not embryonic lethal in zebrafish. However, we observe that maternal c*fap298* mutants have abnormal cell divisions beginning from 4-cell stages to ~256 cell stages, likely as a result of mis-oriented, asynchronous mitotic spindles, yet embryos recover by MZT. To further investigate the role of CFAP298 in tissues without motile cilia, we developed a *Cfap298* floxed allele for tissue-specific knockouts in mouse. Upon conditionally removing *Cfap298* function in the developing mouse epidermis using Tg(RT14-cre)1Efu, we observed skin formation to be severely impaired in P0 pups, leading to post-natal death by dehydration. From E15.5 -E17.5 stages, we observed embryos to have reduced stratification, reduced skin barrier function, and increased cell death, likely leading to impairment of skin morphogenesis. We are currently investigating whether effects on spindle orientation and function may underlie the non-motile cilia associated phenotypes we observe in our different systems. Overall, our work uncovers novel functions for *Cfap298* in vertebrate development that extend beyond cilia motility.

1229S **The KAT6A-NCOA2 (MOZ-TIF2) fusion protein drives aberrant gene expression in a mouse model of Acute Myeloid Leukemia (AML)** Daniel W Sullivan[1,2], Anne Smolko[1,2], Naomi Olsen[3,4], Florian Perner[3,4], Jonathan Baell[5], Scott Armstrong[3,4], Mtizi Kuroda[1,2] [1]Genetics, Brigham and Women's Hospital, [2]Harvard Medical School, [3]Dana Farber Cancer Institute, [4]Boston Children's Hospital, [5]Monash University

Many cancer types exhibit aberrant genome regulation due to improperly activated chromatin, which has made targeting

chromatin modifiers a potential strategy in cancer therapeutics. KAT6A (MOZ) is a histone acyltransferase that is required for normal embryogenesis and hematopoiesis and is recurrently fused to transcriptional co-activators Ep300, CREBBP (CBP), or NCOA2 (TIF2) to drive Acute Myeloid Leukemia (AML). Two major goals of our research are to understand how MOZ leukemic fusion proteins alter transcription to drive leukemia and how MOZ normally regulates gene expression during development. Using pharmacological inhibition and targeted protein degradation, we show that MOZ enzymatic activity and the MOZ-TIF2 fusion protein are necessary for indefinite proliferation in a murine primary leukemia cell line expressing a human MOZ-TIF2 transgene. Inhibition of MOZ activity results in decreased histone acylation, including the understudied Histone 3 Lysine 23 propionylation (H3K23pr) modification. By integrating chromatin binding profiles of MOZ-TIF2 and H3K23pr with RNAseq datasets, we show that MOZ-TIF2 directly targets a set of developmental genes that are potential drivers of AML. In parallel, we are investigating MOZ function in a normal developmental context. For these studies we are utilizing murine embryonic stem cells as a model system, aiming to (A) epitope tag endogenous MOZ to map its genome localization pattern, and (B) determine how MOZ inhibition and degradation affect genome-wide acylation levels and gene expression. Overall, our findings will complement our previous analyses in the MOZ-TIF2 leukemia model to provide a greater understanding of MOZ function in both normal and disease states.

1230S **Role of *Mirc5* (aka *Mir 290-295)* and *Mirc20* (aka *Mir-302/367)* Clusters in Testicular Germ Cell Tumor Development** Harlie Cope, Olga Medina-Martinez, Matthew Gonzalez, Denise Lanza, Ronald Parchem, Jason Heaney Molecular and Human Genetics, Baylor College of Medicine

Testicular germ cells tumors (TGCTs) originate during development as a result of germ cells failing to complete differentiation. Understanding how these tumors originate and develop is important as TGCTs are the most prevalent form of solid cancer in young men, and the incidence has increased in recent years. Sequencing studies show that several miRNA clusters are upregulated in both tumor samples and serum from patients with TGCTs, including the *MIR371* family, aka *MIR371-373* and *MIR302* family, aka *MIR302/367* clusters. These clusters are important regulators of pluripotency in early embryonic development, with the *Mirc5* aka *Mir290-295* (murine equivalent of human *MIR371-373*) expressed during naïve pluripotency and *Mirc20* aka *Mir302/367* expressed slightly later when the embryo is in a primed pluripotent state. We evaluated the expression of these miRNA clusters and their effects on TGCT development in the M19 mouse, which has a high rate of spontaneous testicular tumor development. In this model embryonal carcinoma (EC) cells, which are the precursors to tumors seen in adulthood, are identifiable at embryonic day 15.5 (E15.5). Using a *Mirc5* reporter mouse, we have shown that germ cells express *Mirc5* as early as E12.5, and that this expression is maintained through post-natal day 2 (PND2), as well as in EC foci seen at E15.5 and E16.5. Using a *Mirc20* knockout/reporter line, we visualized expression of the *Mirc20* cluster at different embryonic timepoints in germ cells and EC foci. Expression of the *Mirc20* cluster is not detectable in germ cells using this reporter line; however, it is expressed in EC cells as early as E15.5 through PND2. The timing of the *Mirc20* GFP reporter expression in EC foci corresponds with immunofluorescent staining for OTX2, a transcription factor expressed in primed pluripotent EC cells. We assessed EC foci/tumor incidence in *Mirc20* heterozygous mice at E16.5 and at 8-12 weeks of age. At E16.5, 88% of heterozygous embryos had EC foci compared to 100% of wildtype littermates (N=8 per genotype, P=0.3017, Chi-square). In the 8-12 week old adults, 42% of heterozygotes had tumors compared to 76% of wildtype littermates (Het N=26, WT N=21, P=0.020, Chi-square). This reduction in tumor incidence along with the correlation of miR-302/367 and primed pluripotency marker OTX2 suggests that *Mirc20* expression may be important in maintaining the proliferative state needed for the EC foci to fully differentiate into teratomas. We will continue to characterize the *MIrc5* cluster in later EC cells. As homozygous knockout of each cluster is embryonic lethal at stages prior to EC cell development, we have generated conditional knockout lines for each cluster to determine the impact of homozygous knockout specifically in the germ cells on tumor incidence. We plan to isolate wild-type *Mirc5* knockout and *Mir302a/b/c/d* knockout germ cells to extract RNA for differential expression analysis of mRNA targets.

1231S **CRISPR Induced Overexpression of Placental *Igf-1* Causes Sex Specific Changes in Placental and Fetal Development in Mice** Annemarie Carver[1], Robert Taylor[2], Faith Fairbairn[2], Hanna Stevens[2] [1]Psychiatry, University of Iowa, [2]University of Iowa

Insulin-like growth factor 1 (IGF-1) is an essential hormone that is primarily produced in the placenta prior to birth. IGF-1 has a significant impact on fetal growth directly but also through other placental functions it regulates at the transcriptional level. IGF-1 levels impact the expression of IGF binding proteins, other growth factors, and nutrient transporters all localized in the labyrinth zone or transport region of the placenta. Altering placental IGF-1 may have significant effects on the developing fetus, particularly tissues that require GF-1 for growth of the brain and all elements of the body. We hypothesized that overexpression of placental *Igf1* would promote expression of its downstream targets subsequently accelerating overall growth of the fetus. We created a placental targeted overexpression of *Igf1* using an *in utero* CRISPR activation technique developed in our lab and assessed with qPCR and ELISA, as well as placental and fetal growth as developmental outcomes. Interestingly, we found that males and females responded differently to this manipulation. At E14 and E18, female overexpression placentas displayed the expected increased placental *Igf1* gene expression and protein levels compared to controls. Males did not show

these changes with the overexpression manipulation but had higher placental *Igf-1* than control females generally which may reflect a ceiling effect limiting the impacts of the CRISPR manipulation. Furthermore, males with placental CRISPR activation had increased fetal body IGF-1 protein at E14. This suggested a previous temporary placental overexpression as a source for higher E14 body IGF1 that was then downregulated in placenta to control levels by E14. In females, IGF binding proteins, placental growth factor, and amino acid transporter placental gene expression was higher after *Igf1* overexpression. Male placentas after the CRISPR activation showed only decreases in these outcomes. Despite these changes, placental size and zone areas were not altered in either sex. Female body mass was larger at E14 but unchanged at E18, while male body mass showed no change at E14 but was decreased at E18. These sex-specific growth changes were consistent with the sex-specific changes found in other placental genes, regulated by placental IGF-1 levels. Overall, these studies demonstrate how male and female placental *Igf1* may be differentially constrained, affecting its regulation of gross fetal development.

1232S **Pumilio proteins interact with UPF1 to promote degradation of a subset of pumilio-target mRNAs in mouse embryonic stem cells** Yuedong Huang, Yiying Yang, Haifan Lin Yale University

Post-transcriptional regulations play crucial roles in mammalian embryogenesis yet have not been well studied. Our lab discovered that pumilio RNA-binding family member 1 (PUM1) and PUM2, the two murine members of the RNA-binding Pum protein family, are essential for early embryogenesis and embryonic stem cell (ESC) function. To explore the underlying mechanism, we identified 543 direct mRNA targets of PUM1 and 508 direct mRNA targets of PUM2 in mouse ESCs by enhanced crosslinking and immunoprecipitation (eCLIP). Among them, 330 mRNAs are PUM1-PUM2 common targets, including key pluripotency factors such as *Sox2*, *Nanog*, *Tbx3*, and *Esrrb*. RNA-seq of *Pum1*-knockout (*Pum1*-KO), *Pum2*-KO, *Pum1/2*-DKO, and wild-type ESCs before and after inducing differentiation revealed that, among the 107 Pum-target mRNAs down-regulated in wild-type ESCs during differentiation, the down-regulation is overall reduced in *Pum*-KO ESCs. This reduction is especially significant for 42 mRNAs, which includes mRNAs of pluripotency factors such as *Tbx3*, *Nanog*, and *Esrrb*. These results indicate that PUM1 and PUM2 may promote the degradation of these 42 target RNAs. In addition, for the 58 PUM-target mRNAs that are up-regulated in wild-type ESCs during differentiation, the up-regulation is variably affected in *Pum*-KO ESCs. This implicates that PUM proteins can exert different regulatory effects on different targets. To investigate how PUM proteins mediate target mRNA degradation, we performed immuno-precipitation and mass spectrometry of PUM1 protein in ESCs and identified UPF1, a key factor in nonsense-mediated decay (NMD) pathway, as a novel protein partner for PUM1. Disrupting the UPF1-interaction region of PUM1 abolished its regulatory effect. This result indicates that PUM1 and UPF1 function together to degrade target mRNAs. Overall, our work reveals a novel and complex mechanism of gene regulation at post-transcriptional levels that is essential for ESC pluripotency.

1233S **Genetics and genomics in planarians and geckos** Longhua Guo University of Michigan

The decreasing cost and increasing power of sequencing allowed unprecedented accessibility to the secrets of genomes from a wide range of species. In my laboratory, we combine traditional genetics and modern genomics to study traits in two non-traditional laboratory animal models, the planarians, and leopard geckos. For planarians, we use established inbred lines to build laboratory mapping populations. For leopard geckos, we collaborate with breeders to collect DNA samples from a large collection of breeding populations. These approaches allow us to identify casual genotype and phenotype relationships in non-traditional animal models.

1235S **Defining a role for Hedgehog signaling in zebrafish left-right patterning** Cullen B Young, Rebecca D Burdine Molecular Biology, Princeton University

Underneath the exterior of a bilaterally symmetric vertebrate is an internal body plan characterized by distinct left-right (LR) asymmetries in organ structure and position. LR asymmetry arises at a structure referred to as the left-right organizer (LRO) that is required for proper LR axis specification. Errors in LR axis specification result in birth defects such as heterotaxy, and congenital heart disease. While the initial symmetry breaking event at the LRO varies across vertebrates, amplification of the transforming growth factor β (TGFβ) morphogen Nodal at the LRO and followed by asymmetric Nodal signaling is highly conserved. The Hedgehog (Hh) pathway, the first pathway to be implicated in vertebrate LR patterning, is known to be required for asymmetric organ development in a variety of organisms such as mice and chick. However, its specific influence on *nodal* has been controversial due to temporal adaptation mechanisms in the absence of Hh activity and Hh effects on morphological structures such as the midline which indirectly impacts asymmetry of *nodal* expression. To address this, we sought to investigate whether Smoothened-mediated hedgehog signaling is required for LR patterning in zebrafish. Surprisingly, while all Hh pathway components are expressed in or around Kupffer's vesicle (KV), the zebrafish LRO, a requirement in LR patterning has yet to be described. To avoid the confounding variables brought by genetic mutants and exogenous over-expression, we turned to pharmacological manipulation using agonists and antagonists of Smoothened (Smo), the key transducer of the pathway during critical periods of LR development to delineate any temporal requirements.

-Strikingly, we find a minimal period where embryos are sensitive to Smo perturbations, preventing the establishment of proper asymmetric markers. Our findings suggest that Smoothened-mediated Hh signaling has a previously uncharacterized role in zebrafish LR patterning and requires further mechanistic investigation. Overall, our work highlights the importance of modulating signaling strength and duration during embryonic development and provides insight to the molecular players instructing asymmetric organogenesis.

1236S **Investigating the Role of the Adaptor Protein Enkurin in Zebrafish Left-Right Patterning** Billie M Reneker[1], Vanessa Gonzalez[1], Triveni Menon[2], Rebecca Burdine[1] [1]Molecular Biology, Princeton University, [2]HHMI Janelia Research CampuS

Vertebrates are externally symmetric but have critical internal asymmetries in organ placement and patterning. Asymmetries along the left-right (LR) body axis are established within embryonic structures known as LR organizers, which in zebrafish is Kupffer's vesicle (KV). Current models propose that motile cilia within KV generate a leftward flow of extracellular fluid that is sensed by KV cells via the cation channel complex Polycystin 2 (Pkd2) and Polycystin 1 Like 1 (Pkd1L1), leading to an increased flux of Ca^{2+} in cilia. Increased ciliary Ca^{2+} would then signal through unknown mechanisms to culminate in asymmetric posttranscriptional regulation of the Nodal inhibitor, *dand5*, which would restrict Nodal signaling to the left side of the embryo to accomplish proper LR patterning. However, the precise targets of flow and/or calcium signaling that connect the Polycystin complex to regulation of *dand5* mRNA are entirely unknown. To identify these effectors, we carried out single-cell RNA sequencing of zebrafish KV cells. From this dataset I identified the gene *enkurin* as highly enriched in KV cells when *dand5* asymmetry is being established. Based on published work in Mouse sperm flagella (Sutton et al., *Dev Biol*. 2004; Jha et al., *Can. J. Physiol. Pharmacol*. 2015.), I hypothesize Enkurin acts as an adaptor protein to link Pkd2 to downstream Ca^{2+} signaling effectors which regulate the downregulation of *dand5* in response to flow. To test my hypothesis, I have generated *enkurin* CRISPR/Cas9 mutants and will present my characterization of the mutant phenotypes. I am currently testing binding of Enkurin to Pkd2, Calmodulin, and other proteins involved in the LR patterning pathway and determining localization of Enkurin within KV cells. My work will provide insight into the mechanisms downstream of flow in the zebrafish LRO that controls *dand5* asymmetric expression.

1237S **Urotensin Signaling Controls Development of Muscle and Spine Morphology** Johnathan O›Hara-Smith, Elizabeth Bearce, Samuel Bertrand, Rachael Giersch, Daniel Grimes Biology, University of Oregon

As animals mature, the body grows rapidly and must properly scale its tissues and organs to maintain the right shape. Failure to properly develop the musculoskeletal system can result in scoliosis, that is the breakdown of the morphology of the spine. Idiopathic scoliosis (IS), defined by acquisition of spinal curves during adolescence in the absence of explicative neuromuscular or vertebral structure defects, is highly prevalent, affecting about 3% of humans, yet poorly understood. Using zebrafish genetic models of IS, we have identified 2 cyclic neuropeptides, the Urotensin II-related peptides 1 and 2 (Urp1 and Urp2), which are secreted from within the spinal cord and control spinal morphology as well as muscle development. When Urp1 and Urp2 are deleted, spine morphology breaks down severely during juvenile growth, producing scoliosis-like phenotypes. To understand the molecular developmental mechanisms leading to this spinal dysmorphology, we performed bulk RNA-sequencing of Urp1/Urp2-deficient embryos, identifying multiple dysregulated transcripts encoding proteins critical for the basic function of muscle. Confocal microscopy to observe embryonic and larval somitic musculature revealed subtle structural defects including cavities in muscle tissues as well as waviness of muscle fibers. Additionally, spinal dysmorphology occurred when we mutated the muscle-expressed Urp1/Urp2 receptor, Uts2r3. We hypothesize Urotensin signaling controls spine morphology by controlling the way trunk muscle tissue grows and matures, where mismatched growth or maturation with vertebral structures, organs, and other tissues results in breakdown of spine morphology.

1238S **Optic Fissure Morphogenesis and the Mechanisms Driving Coloboma** Ryan Clough, Keith Carney, Kristen M Kwan Human Genetics, University of Utah

Tissue morphogenesis is a critical process that creates the three-dimensional tissue shape necessary for its proper function, and birth defects can arise when these processes go awry. Coloboma, an ocular birth defect and a significant cause of childhood blindness worldwide, manifests due to structural developmental defects in the optic fissure, a transient embryonic eye structure. The optic fissure forms as two tissue margins at the ventral face of the embryonic eye and optic stalk, creating an avenue for blood vessels to supply the developing eye as well as for retinal axons to exit and innervate the brain. After the optic fissure forms, the margins subsequently fuse, enwrapping these blood vessels and axons. Defects in either the formation or fusion of the optic fissure can lead to coloboma. Coloboma research has heavily focused on mechanisms of optic fissure fusion, but morphogenetic studies of optic fissure formation remain comparatively unexplored. Previous work has uncovered the developmental origin of the cells that form the optic fissure; however, little is known of the cell morphology changes and movements underlying optic fissure formation. Here, we combine multidimensional imaging of zebrafish embryos as well as custom software tools developed in our lab to segment and track cells in 4D to characterize the cell movements and cell morphologies driving optic fissure formation.

Our preliminary analyses reveal some potentially surprising behaviors: cells populating the optic fissure margins may undergo lateral constriction, suggesting an unexpected invagination, or folding event that is responsible for optic fissure formation. Moving forward, we aim to comprehensively define the cell behaviors directing this process, which will serve as a foundation to understand the molecular mechanisms controlling optic fissure formation and its disruption in coloboma. We previously found that overactive Hedgehog (Hh) signaling, as found in the zebrafish *ptch2* mutant, disrupts optic fissure formation. In the future, we will determine the specific molecular mechanism by which overactive Hh signaling, and its downstream effectors, alter the morphogenetic processes underlying optic fissure formation.

1239S **The role of chemokine signaling in the projection of habenular axons in zebrafish** Catherine Stress, Ryan Anderson, Sara Roberson Applied Science, Bluefield State University

The dorsal habenulae (dHb) are a conserved, bilateral region of the forebrain responsible for behaviors such as fear, anxiety, and comprehension of reward versus punishment. Axons of dHb neurons project posteriorly to synapse with neurons of the interpeduncular nucleus, an unpaired region of the midbrain. During development of the dHb, its precursor cells express the *chemokine (C-X-C motif) receptor 4b* (*cxcr4b*). Active chemokine signaling through Cxcr4b is detected at the distal ends of newly developing axons. Two chemokine ligands, *chemokine (C-X-C motif) ligand 12a* and *12b* (*cxcl12a* and *cxcl12b*) are expressed in the region of the developing dHb. Previous work has shown loss of either *cxcr4b* or *cxcl12a* results in the defasciculation and anterior projection of dHb axons. A similar axon phenotype is detected in a mutant where overexpression of ligand *cxcl12b* is observed in the region of the developing dHb. The individual roles of *cxcl12a* and *cxcl12b* in directing dHb axons to their midbrain target is not fully understood. Relative expression patterns of the two ligands and axon phenotypes in zebrafish larvae lacking *cxcl12b* are currently being explored. Preliminary data suggests that global overexpression of *cxcl12a* does not phenocopy the loss of *cxcl12a* or overexpression of *cxcl12b*. Together, this suggests that *cxcl12a* and *cxcl12b* play distinct, non-redundant roles in directing dHb axons to their midbrain targets.

1240S **The Role of *tango6* in Embryonic Zebrafish Development** Sydney Versen[1], Hannah Herron[1], Kelli Carroll[2] [1]Austin College, [2]Wofford College

The Undiagnosed Diseases Network (UDN) is a collection of clinicians and researchers that diagnose individuals with rare or previously uncharacterized diseases. Mutations in *TANGO6* were identified in a child with neurological and cardiovascular defects. While data from The International Mouse Phenotyping Consortium indicated that TANGO6 is required for development, the function and expression patterns of TANGO6 are largely unknown. To understand the role that *tango6* plays in development, we utilized embryonic zebrafish to analyze its quantitative and spatial expression. *tango6* is expressed most highly between 0 and 12 hours post fertilization (hpf) but continues to be expressed at low to moderate levels from 24-120 hpf. *In situ* hybridization demonstrated that *tango6* is broadly expressed in zebrafish embryos from the 256-cell stage through early somitogenesis. In older embryos, *tango6* showed more restricted localization with expression in the brain beginning at 24 hpf and in a specific region of the lower jaw by 72 hpf. Furthermore, by 96 hpf, *tango6* is expressed in the developing gastrointestinal system and swim bladder. Preliminary data of mosaically edited *tango6* knockdowns generated using CRISPR (CRISPANTS) show a transient accumulation of blood around the gut by 120 hpf, suggesting defects in gut morphogenesis or vascular permeability. Additionally, *tango6* CRISPANTS present with craniofacial malformations and potential liver abnormalities. Intriguingly, knockdown of *tango6* using a morpholino showed a more dramatic phenotype, with high rates of lethality and broad morphological defects, indicative of a potential maternal mRNA contribution. In total, these data suggest that *tango6* is involved in early zebrafish development, and further analysis of knockdowns and spatial expression patterns is underway to determine the precise role of *tango6* in development and disease.

1241S **Maternal Osbpl7 factor controls cleavage furrow formation during the embryonic first cell division** Ingrid del Pilar Pinto-Borguero[1,2], Natalia Benavente-Cabrera[1], Felipe Aguilera[3], Mary C. Mullins[4], Ricardo Fuentes[5] [1]Cell biology, Universidad de Concepcion, [2]Cell and developmental biology, University of Pennsylvania, [3]Biochemistry and Molecular Biology, Universidad de Concepcion, [4]Cell and Developmental Biology, University of Pennsylvania, [5]Cell Biology, Universidad de Concepcion

After fertilization, the initial stages of embryonic development involve the orchestration of the first round of cell division, a highly regulated process guided by genetic products—RNAs and proteins—loaded by the mother and stored into the oocyte during oogenesis. Despite the increasing awareness of the pivotal role maternal products play in vertebrate development, only a limited number of them have been discovered.

We have isolated the maternal-effect zebrafish mutant that harbors a mutation in the *osbpl7* gene, which encodes a lipid transporter associated with the oxysterol-binding protein family. Employing techniques such as microscopy, immunostaining, and bioinformatic analyses, we found that the mutant phenotype completes meiosis post-fertilization. However, it exhibits notable aberrations in egg activation and cleavage furrow formation during both initial and subsequent cell divisions.

Furthermore, there is plasma membrane instability leading to subsequent cell lysis, indicating that the mutation is highly penetrant and lethal in the offspring of a mutant female fish. Our initial studies of differentially expressed genes also reveal that the mutation impacts on factors related to lipid homeostasis and cytoskeletal rearrangement.

This research not only establishes a novel link between a member of the oxysterol lipid transporter family and the first embryonic cell division but also identifies it as a pivotal maternal factor crucial for early developmental processes. Ongoing studies are in progress to understand the mechanisms of how Osbpl7 participates in the meiosis-to-mitosis transition.

1242S **Systematic analysis of temperature-sensitive alleles uncovers new functions for essential genes in yeast filamentous growth** Atindra N Pujari[1], Zhijian Li[2], Ankita Priyadarshini[1], Deanna Williams[1], Dale Climie[2], Sondra Bahr[2], Helena Friesen[2], Michelle Li[1], Joshua Oken[1], Brenda Andrews[2], Charles Boone[2], Paul J Cullen[1] [1]Biological Sciences, State University of New York at Buffalo, [2]Terrence Donnelly Center for Cellular and Biomolecular Research, University of Toronto

Through the process of differentiation, cells specialize into unique types with distinctive shapes and specific functions. Many fungal species, including pathogens, differentiate to adherent, pseudohyphal, and hyphal cell types, which are collectively referred to as filamentous growth, in response to environmental cues. Certain strains of baker's yeast *Saccharomyces cerevisiae*, such as the Σ1278b background, also undergo filamentous growth. Studying filamentous growth in yeast has facilitated the identification of many genes and regulatory pathways that control these behaviors. A group of genes yet to be systematically analyzed for their role in filamentous growth are those that perform essential functions. We generated a collection of temperature-sensitive (ts) alleles in 325 essential genes in the Σ1278b strain to interrogate their roles in filamentous growth. Ts mutants were examined at semi-permissive temperatures for phenotypes in different aspects of filamentous growth, such as invasive growth, pseudohyphal growth, and biofilm/mat formation. More than half the alleles tested showed phenotypes by at least one assay. New connections to filamentous growth and splicing, kinetochore assembly, and chromosome segregation were identified. We also found that the Wiskott-Aldrich Syndrome protein homolog Las17p regulated the Cdc42p-dependent MAP kinase pathway (the fMAPK) that controls filamentous growth. Las17p was required for the localization and levels of Sho1p, a tetraspan sensor of the fMAPK pathway. Our results, including identification of new roles for essential genes in cell differentiation and variation in essential processes across populations, may improve the understanding of essential gene functions in eukaryotes.

1243V **The AP-1 clathrin adaptor complex differentially regulates Notch signaling** Tatsuya Kato[1,2], Olga Skorobogata[1,2], Haojun Zhu[1,2], Edouarda Taguedong[1,2], Christian Rocheleau[1,2,3] [1]Research Institute of the McGill University Health Center, [2]Department of Anatomy & Cell Biology, McGill University, [3]Department of Medicine, McGill University

Notch signaling relies on interactions between transmembrane Notch receptors and ligands expressed on neighboring cells to influence cell differentiation and proliferation. Consequently, its roles in diseases such as cancer are varied, and it is vital to further understand how Notch signaling is regulated in different tissues to shed light on their pathologies. *C. elegans* vulval development is a powerful system to study tissue-specific Notch signaling regulation since it is controlled by Notch-dependent cell fate specifications in the gonad and vulval epithelium. A somatic gonad precursor cell with low LIN-12/Notch activity is specified to become the anchor cell (AC). AC function is critical for subsequent induction of vulval precursor cells (VPCs), two of which are specified to the secondary fate through high LIN-12/Notch activity. Modulations of Notch signaling cause observable developmental defects such as ectopic secondary fate inductions or lack of VPC inductions called the vulvaless phenotype. AP-1 is a conserved clathrin adaptor complex known to regulate transmembrane protein sorting from the Golgi to the plasma membrane. We previously reported that double mutants of the AP-1 subunit *unc-101/ap1m1* and the Rab GTPase *rab-7/rab7a* exhibited ectopic VPC inductions with secondary fate morphologies, suggesting a role of AP-1 in Notch signaling.

To investigate, I analyzed effects of the *unc-101(sy108)* loss-of-function mutant in a *lin-12(n302)* background. *lin-12(n302)* is a partial gain-of-function mutant which inhibits AC specification but does not ectopically induce secondary fates, resulting in a vulvaless phenotype. I observed that *unc-101(sy108)* suppressed the *lin-12(n302)* vulvaless phenotype through restoration of the AC fate, indicating that AP-1 activity promotes Notch signaling in the AC. Interestingly, *unc-101(sy108); lin-12(n302)* animals also exhibited ectopic secondary fate inductions in the VPCs, suggesting that UNC-101/AP1M1 inhibits LIN-12/Notch in the VPCs. Furthermore, expression analyses of a LIN-12/Notch reporter showed that AP-1 promotes and inhibits LIN-12/Notch membrane expression in the AC and the VPCs respectively. These results point to differential Notch signaling regulation by AP-1 in the somatic gonad and vulval epithelium. I am currently testing if AP-1 differentially regulates Notch signaling through tissue-specific LIN-12/Notch sorting and/or cell polarity maintenance.

1244V **Understanding the development and function of Drosophila descending neuron controlling motor programs** Alex Le[1], Kristen Lee[2], Chris Doe[2], Matthew Clark[3] [1]Bucknell University, [2]Institute of Neuroscience, University of Oregon, [3]Department of Neuroscience, Bucknell University

While research on Drosophila melanogaster has yielded substantial knowledge about neuronal differentiation, synaptogenesis, and more, there's limited understanding of the specific genes forming the neural circuit during metamorphosis. Our study investigates the roles of transcription factors (TFs) and cell-surface molecules (CSMs) in the moonwalking descending neurons (MDN) circuit, pivotal for fruit flies' locomotion. Using techniques like genetic screening, optogenetics, and confocal microscopy, we examined the impact of different genotypes on the MDN circuit post-metamorphosis and aimed to reconstruct the descending neurons (DNs) circuits. Our findings highlight that specific TFs, CSMs, and some known descending neurons are integral to MDN circuit formation, as observed through the altered locomotion behaviors and atypical neuronal morphology. Considering the genetic parallels between fruit flies and humans, this research holds significant ramifications for developmental neurobiology. Furthermore, the insights offer potential avenues for enhanced human disease screening, underscoring the interplay between genetics, neural connectivity, and health.

1245V **Arrowhead, a LIM homeodomain protein plays critical role in Notch mediated wing and neuron development in *Drosophila*** Jyoti Singh, Ashim Mukherjee Molecular and Human Genetics, Banaras Hindu University

Notch pathway is an evolutionarily conserved signaling pathway that tends to influence an astonishing array of cell fate decisions in different developmental contexts. To identify novel effectors of Notch signaling, the whole transcriptome of the *Drosophila* wing imaginal discs was analysed in which an activated form of Notch was overexpressed. Among the candidate genes, a LIM-homeodomain protein encoding gene, *Arrowhead* (*Awh*) was identified as a novel candidate which plays an important role in Notch-mediated developmental events. A strong genetic interaction between the alleles of Awh and Notch pathway components was also observed. Immunocytochemical analyses revealed that Awh overexpression downregulates Notch targets, Cut and Wingless (Wg). This reduction of targets is regulated through the downregulation of the ligand, Delta without altering Notch receptor levels. As a consequence the expression of Wg effector molecule Armadillo, and its downstream targets Senseless and Vestigial also gets downregulated. Further, *Awh* overexpression resulted in ectopic expression of *engrailed*, a segment polarity gene in anterior region of wing disc, leading to duplication of the wing. We also observed that Awh overexpression significantly rescues neuronal defects caused by activated Notch. Interestingly, activated Notch inhibits Awh activity, indicating a regulatory loop between Awh and Notch. In addition, the defects caused by Awh overexpression were significantly rescued by Chip, a LIM-interaction domain containing transcriptional co-factor. The LIM domain of Awh is crucial for the Awh-Chip interaction. The present study highlights the feedback regulation between Awh and Notch and elucidate their significance in *Drosophila* wing and neuronal development.

1246V **Epigenetic Avoidance of *Drosophila* Egg Chambers with an Excessive Number of Oocytes** Laura Castro, Talia Feijao, Joao Rede, Rui Gonçalo Martinho University of Aveiro

Oocyte determination occurs in many organisms within a germ line cyst, a multicellular structure composed of interconnected germ cells. In *Drosophila*, mouse and many other organisms, each cyst forms a single oocyte, whereas the remaining interconnected cells of the cyst differentiate as supporting nurse cells, which will ultimately die by programmed cell death.

Interestingly, early on, multiple cells of the cyst enter meiosis, but only one is capable of remaining in meiosis and avoiding a nurse cell fate as the cyst differentiates. This suggests that the mechanisms required for oocyte determination are likely to go hand-in-hand with poorly understood restriction mechanisms that avoid the formation of multiple oocytes within a single germ line cyst.

Recently, we and others observed that Polycomb-group proteins (PcG proteins) have a critical function in the repression of early prophase I genes during mid/late prophase I (PMID: 32773039 and 36215502). We therefore hypothesized that such transcriptional repressive activity within the female germ line cyst is likely to contribute for the avoidance of *Drosophila* egg chambers with supernumerary oocyte formation.

In order to test this hypothesize, we screened for genes whose misexpression, in the context of defective Polycomb silencing, could facilitate the formation of *Drosophila* egg chambers with excessive number of oocytes. We identified a chromatin-related protein whose expression up-regulation dramatically increase the frequency of *Drosophila* egg chambers with multiple oocytes. Interestingly, and although the viability of such egg chambers is reduced, we could easily detect egg chambers with multiple oocytes until mid/late oogenesis (stage 9).

We will present our ongoing work, where we try to mechanistically understand the way supernumerary oocyte formation is avoided in *Drosophila*. Our working hypothesis is that highly conserved oocyte-fate restriction mechanisms are likely to exist in the female germ line cyst.

1247V ***ztf-16* is a novel heterochronic modulator that opposes adult cell fate in dauer and non-dauer life histories in *Caenorhabditis elegans*** Mark A Hansen[1], Anuja Dahal[1], Taylor A Bernstein[1], Chani Kohtz[1], Safiyah Ali[1], Aric L Daul[2], Eric

Montoye[1], Ganesh P Panzade[3], Amelia F Alessi[4], Stephane Flibotte[5], Marcus L Vargas[2], Jacob Bourgeois[1], Campbell Brown[1], John K Kim[4], Ann E Rougvie[2], Anna Zinovyeva[3], Xantha Karp[1] [1]Central Michigan University, [2]University of Minnesota, [3]Kansas State University, [4]Johns Hopkins University, [5]University of British Columbia

To increase the chance of survival in adverse environments, *C. elegans* can enter dauer diapause, a stress-resistant, developmentally arrested stage adopted after the second larval molt. If dauer larvae find favorable conditions, they can exit dauer and complete development. To study the effect of dauer diapause on development, we focus on the epidermal seam cells. Seam cells are multipotent during larval development but differentiate in adults, a process that is regulated by the heterochronic genes. Interestingly, many heterochronic genes are required only during non-dauer development, suggesting that novel genes may control post-dauer seam cell development. To identify such genes, we conducted a genetic screen for mutants displaying precocious expression of the adult-specific *col-19p::gfp* marker in post-dauer larvae. We found that *ztf-16*, encoding a C2H2 zinc finger transcription factor, is required to prevent precocious *col-19p::gfp* expression beginning in the L1 stage in both dauer and non-dauer life histories. Comparative mRNA-seq analysis also identified 306 differentially expressed genes between wild-type and *ztf-16(-)* larvae. In non-dauer development, *col-19* expression is regulated by a genetic pathway including the *let-7* microRNA, the LIN-41 RNA binding protein, and the LIN-29 transcription factor that directly activates *col-19* transcription. We used genetic and molecular analyses to place *ztf-16* within this pathway. Expression of *ztf-16::gfp* was strongly upregulated in *let-7(-)* mutant larvae, indicating that *ztf-16* acts downstream of *let-7*. Genetic epistasis experiments further supported this interpretation. However, mutation of the *let-7* site in the *ztf-16* 3'UTR did not affect *ztf-16::gfp* expression, suggesting that *let-7* regulates *ztf-16* indirectly. *lin-41* is a target of *let-7*, and we hypothesized that *lin-41* may mediate regulation of *ztf-16* downstream of *let-7*. Surprisingly, RNAi of *lin-41* did not suppress the upregulation of *ztf-16::gfp* and in fact enhanced this phenotype. Finally, *lin-29* was not required for precocious *col-19p::gfp* expression in *ztf-16(-)* larvae, suggesting that additional players are involved in regulating *col-19p::gfp* expression. Taken together, our work describes a novel heterochronic regulator that functions after dauer diapause.

1248V **Drosophila REV7 Promotes Genome Stability Independently of Pol ζ** Lara R Maggs, Mitch McVey Tufts University

REV7 is best known as a component of DNA translesion polymerase ζ. This specialized polymerase is involved in synthesis past DNA lesions at replication forks and single-strand gaps during postreplication repair. It's also a subunit of other protein complexes involved in genome stability, including the Shieldin complex, which regulates double-strand break repair pathway choice in mammals, and the mitotic spindle assembly checkpoint complex, which prevents premature onset of anaphase. REV7, a HORMA domain protein, uses its characteristic safety belt region to interact with other proteins in these complexes.

Although the Drosophila REV7 protein shares the same HORMA domain as its orthologous counterparts, it remains unknown whether it functions beyond its canonical role as a Pol ζ constituent. To test this, we used mutant stocks with transposon insertions in the REV7 coding sequence, along with CRISPR-generated mutants, and compared them to flies lacking REV3, Pol ζ's catalytic subunit. We assessed the mutants for their response to innate endogenous damage, including adult viability, hatching rate, and eggshell abnormalities. Strikingly, REV7 loss decreases homozygote viability, impairs hatching rate, and hinders eggshell development to a greater extent than REV3 loss. Additionally, we conducted mutagen sensitivity assays with the alkylating agent methyl methanesulfonate (MMS) to evaluate these mutants' response to exogenous damage. MMS induces replication-blocking adducts that, if not swiftly addressed, cause deleterious outcomes like fork collapse or aberrant checkpoint activation. At all MMS concentrations tested, REV7 loss caused greater hypersensitivity than REV3 loss.

The divergent data obtained using rev3 and rev7 mutants indicates the observed defects are not solely due to Pol ζ loss. Instead, these data suggest REV7 interacts with proteins other than REV3 to coordinate damage response and to promote proper development. We are currently conducting proteomic studies to identify REV7's uncharacterized protein interactors. These studies will provide deeper insight into the mechanism(s) by which Drosophila REV7 promotes genome stability in response to endogenous and exogenous damage.

1249V **Single cell genomic strategies for prioritizing candidate genes in *Sox10^{Dom}* Aganglionosis Modifier Intervals** Joseph T Benthal, Justin A Avila, E Michelle Southard-Smith Genetic Medicine, Vanderbilt University

Enteric nervous system (ENS) development requires coordinated gene expression that regulates enteric neural crest-derived cell (ENCDC) migration, enteric gliogenesis, and enteric neurogenesis. Perturbations in genes involved in these initial processes can result in gastrointestinal motility disorders, including Hirschsprung's disease (HSCR). The *Sox10^{Dom}* mouse model of HSCR recapitulates the variable penetrance of the aganglionosis phenotype. To identify the genetic loci that modify severity and penetrance of the *Sox10^{Dom}* aganglionosis phenotype, our group conducted a genome-wide linkage study on *Sox10^{Dom}* F_2 progeny from a cross of C3HeB/FeJ and C57B6/J mice. These strains exhibit differential severity of the *Sox10^{Dom}* aganglionosis phenotype. Five modifier loci intervals were identified from this cross, three of which are novel and

do not coincide with known aganglionosis susceptibility loci or intervals containing genes known to participate in neural crest development. We have since conducted a genetic scan of an extended pedigree of *Sox10^{Dom}* mice which identified 3 additional modifier intervals. We hypothesize that genes within these *Sox10^{Dom}* modifier loci will contain genes or genomic elements that are differentially regulated in ENS progenitors from wild type and *Sox10^{Dom}* fetal intestine and that intervals around these differentially expressed genes will exhibit differential chromatin accessibility. To address this, we have utilized bulk RNA-seq of enteric neuronal progenitor cells including the migrating wavefront, single cell RNA-seq and single nucleus ATAC-seq of fetal enteric nervous system cells, previously mapped modifier intervals, and our updated modifier intervals from the extended pedigree to identify and prioritize the most relevant candidate genes in ENS development. Our approach has identified candidate genes and regulatory elements that are likely to impact the severity of the *Sox10^{Dom}* aganglionosis phenotype and may point to new mechanisms in which SOX10 interacts with genetic elements to produce phenotypes relevant for human disease.

1250V **The mechanism of of head-tail connection in sperm formation** Danielle B Buglak, Kathleen HM Holmes, Brian J Galletta, Nasser M Rusan National Institutes of Health

During spermiogenesis, spermatids undergo dramatic morphological changes to develop into mature sperm. The final architecture of the sperm requires the lateral attachment of the axoneme (sperm tail) to the needle shaped nucleus (sperm head). Secure attachment is mediated by the head-tail coupling apparatus (HTCA) and its failure results in male sterility due to sperm decapitation. How the HTCA forms and remodels over time is not well understood, and the molecular architecture of the HTCA is an ongoing question in the field. Here, we describe a four stage process of HTCA development: 1. Search and capture of the nucleus by the centriole, 2. Centriole attachment to the nucleus, 3. Centriole insertion into the nucleus, and 4. Centriole lateralization. Using structured illumination microscopy, we identified two unique structures at the HTCA during late spermiogenesis: the “centriole cap” and “nuclear shelf”. We found that the centriole cap is associated with centriole insertion into the nucleus, while the nuclear shelf is associated with centriole lateralization. Two important HTCA components, the testis-specific SUN-domain protein Spag4 and the gravitaxis protein Yuri Gagarin, both localize to the centriole cap and nuclear shelf. Furthermore, we found that the centriole cap and nuclear shelf are closely associated with two additional sperm centriole structures, the proximal centriole-like (PCL) and centriole adjunct (CA). Mutant analysis led to a model whereby the PCL acts as an anchor to maintain centriole positioning at the HTCA during late-stage spermiogenesis. Thus, we identify two unique structures in the sperm HTCA and a novel role for the PCL in male fertility. We are currently working to identify a role for the CA at the head-tail connection and to expand the list of components important for HTCA function.

1251V **Sensory neurons and insulin signaling modulate oogenesis and fertilization in *C. elegans*** Shashwat Mishra[1], Mohamed A Dabaja[1], Asra Akhlaq[1], Bianca Pereira[1], Kelsey Marbach[1], Mediha Rovcanin[1], Rashmi Chandra[1], Antonio Caballero Reyes[2], Diana Fernandes de Abreu[2], QueeLim Ch’ng[2], Joy A Alcedo[1] [1]Department of Biological Sciences, Wayne State University, [2]Centre for Developmental Neurobiology, King’s College London

The survival of an animal species, including those of the worm *C. elegans* and of humans, depends on the physiological state of their oocytes. We have recently shown that specific sensory neurons in the worm can promote a diet-dependent early onset of oogenesis and a faster rate of oocyte fertilization. The chemosensory neurons ASJ stimulate early oogenesis onset through the insulin-like peptide (ILP) INS-6 when *C. elegans* are fed a specific type of bacterial diet. In contrast, the olfactory neurons AWA regulate food type-dependent differences in *C. elegans* fertilization rates but have no role in early oogenesis. AWA neurons modulate fertilization at least partly in parallel to insulin receptor signaling, since the insulin receptor DAF2 regulates fertilization independently of food type, which requires ILPs other than INS-6. Thus, our findings suggest that diverse food-derived cues modulate oocyte biology through different neural signals that optimize an animal’s reproductive program in a given environment.

1252T **New models of transcriptional adaptation in *C. elegans*** Yuntao Charlie Song, Vahan Serobyan, Didier Stainier Max Planck Institute for Heart and Lung Research

Transcriptional adaptation (TA) is a newly identified cellular response whereby a mutation in one gene triggers the transcriptional modulation of other genes, named adapting genes, independent of protein feedback loops. TA has been observed in multiple species, including zebrafish, mice, and *C. elegans*, suggesting a conserved underlying mechanism. However, the current TA models in *C. elegans* have several limitations including the lethality of homozygous mutants and multiple isoforms of the mutant gene. Also, the mRNA splicing and degradation factors required for TA diverge between these models. Hence, more models are needed to study TA in *C. elegans*. A distinct feature of TA is the differential expression of adapting genes in the presence of mRNA decay (e.g., PTC alleles) versus in the absence of transcripts of the mutant gene (i.e, RNA-less alleles). Thus, we explored Wormbase to identify candidate genes with existing promoter-less and premature termination codon (PTC) alleles. So far, we have selected 40 genes that meet these criteria for further investigation. Among

these candidates, two genes, *maco-1* and *agmo-1*, are not expressed in the promoter-less alleles while showing reduced expression in the PTC alleles based on our RNA-seq data. Interestingly, among the *maco-1* PTC alleles, the one with highest level of mRNA reduction displays the highest number of potential adapting genes (i.e., differentially expressed in the PTC allele compared with wild type, but not in the promoter-less one). Moreover, the set of potential adapting genes may depend on the precise location of the PTC (i.e., different PTC alleles might be associated with different adapting genes). Currently, we are trying to narrow down the list of potential adapting genes by impairing essential TA factors, such as *ergo-1*, that were identified using existing TA models. In summary, the initial goal of this project is to discover new TA models that should allow further studies on the underlying mechanisms.

1253T **Analyzing chromatin accessibility using ATAC-seq in *C. elegans*** Nadia Sadri Biology, New York University

Investigating the process of dosage compensation in *C. elegans* provides insights into how chromatin is packaged within the nucleus of a cell. The *C. elegans* dosage compensation complex (DCC) is a multi subunit structure that binds to both X chromosomes of hermaphrodite worms and reduces their transcription. Previous research has shown that the DCC preferentially binds to the regulatory elements of active genes and the level of binding correlates with chromosome accessibility measured by ATAC-seq. However, it is unknown whether accessibility mediates DCC binding or if DCC binding controls accessibility .

To address this question, we aim to perform ATAC-seq upon DCC knockdown. Although routine in many other systems, we found that the ATAC-seq protocol in *C. elegans* produces variable signal to noise profiles for nucleosome presence. The variability comes in part due to the nuclei preparation step yielding compromised nuclei. To prepare intact nuclei, we optimized the current protocol, eliminating the harsh grinding step and reducing the number of pestle strokes used to break the larvae apart. We incorporated a nuclei quality check and input estimate step using a hemocytometer to control the ATAC reaction.

To knock down DCC, we tagged the DPY-26 (a subunit of condensin I and DC) using degron-GFP. This was crossed to a TIR1 strain expressed under the control of the eft-3 promoter for auxin-inducible degradation in somatic cells. We will present our progress in using this strain to knock down DCC and perform ATAC-seq to measure the effect of DCC binding on the accessibility of active gene regulatory elements on the X chromosomes. If there is no change in accessibility following the depletion of DPY-26, we can conclude that DCC binding to the active regulatory elements does not control accessibility. If there is an increase in accessibility, we will test if this is a direct cause of DCC depletion or an indirect effect of increased transcription.

1254T **Poly (U) polymerase activity in *C. elegans* regulates expression and tailing of sRNA and mRNA** Leanne H Kelley[1], Ian V Caldas[1], Matthew T Sullenberger[1], Kevin E Yongblah[1], Anoop Iyer[1], Adnan M Niazi[2], Yini Li[1], Patrick M Tran[1], Eivind Valen[2], Yasir H Ahmed-Braimah[1], Eleanor M Maine[1] [1]Biology, Syracuse University, [2]University of Bergen

Uridylation, the addition of uridine to the 3' end of RNAs (U-tailing), is a conserved post-transcriptional modification documented in many organisms to promote mRNA turnover and implicated in regulating small RNA (sRNA) function. Our previous work showed that poly(U) polymerase (PUP) activity is critical for germline development and embryonic viability, especially under temperature stress. Given this developmental role, we identified the sRNA and mRNA targets of the four known *C. elegans* PUPs and determined the effect of U-tail loss on RNA expression. Ultimately, we aim to determine how the PUPs coordinate proper germline development.

We obtained RNA U-tailing data for adult hermaphrodites carrying *pup* null mutations and raised at 22°C. mRNA data were obtained with a Nanopore-based approach and sRNA data with a high-throughput method. mRNA U-tailing is notably reduced only in the absence of all four PUPs. In contrast, most U-tailed sRNAs are targeted by PUP-1, and distinct subsets of those sRNAs are also targeted by PUP-2, PUP-3, or PUP-4. Surprisingly, PUP-4 apparently *limits* U-tailing of a subset of PUP-1 sRNA targets. Moreover, PUPs have distinct functions in promoting sRNA mono- vs oligo-uridylation. Notably, reduced U-tailing correlates with increased expression of some sRNAs and reduced expression of others.

Numerous siRNAs are differentially expressed in *pup* mutants, and they tend to target genes with germline-enriched or ubiquitous expression. We obtained high-throughput mRNA-seq data for comparison with sRNA-seq data. In the adult, PUP loss correlates with sets of elevated and depleted mRNAs whose products are enriched for several functional classes active in the adult germline and/or early embryo. PUP loss in the early embryo correlates with elevated ribosomal protein mRNAs. Intriguingly, down-regulated siRNAs in the adult include a set that target ribosomal protein genes. Perhaps a diminished contribution of maternal siRNAs leads to higher expression of these mRNAs in the embryo. We will expand on these and other consequences of PUP-mediated regulation in our presentation.

1255T **Transcriptional analysis of *xol-1* mutant hermaphrodites reveals changes in developmental plasticity during embryogenesis** Eshna Jash[1,2], Hector Mendoza[1], Anati Alyaa Azhar[1], Zoey M Tan[1], Halle N Esher[1], Gyorgyi Csankovszki[1] [1]Molecular, Cellular and Developmental Biology, University of Michigan - Ann Arbor, [2]Molecular, Cellular and Developmental Biology, University of Michigan

xol-1, a gene located on the X chromosome, is the master regulator of sex determination and dosage compensation in *C. elegans*. *xol-1* is essential for normal development of XO males, where it promotes the expression of male-specific developmental genes. Additionally, *xol-1* inhibits genes important for hermaphrodite development, which include genes related to X chromosome dosage compensation. While *xol-1* is expressed at very low levels in hermaphrodite embryos, it is thought to be turned off at a very early stage, allowing male-specific developmental genes to be repressed. However, several aberrant phenotypes in *xol-1* mutant hermaphrodites suggest that it does have a function in hermaphrodites as well. We present evidence from bioinformatic analysis in wild-type and *xol-1* mutant embryos showing that the male-specific transcriptional program is active during early hermaphrodite embryogenesis. Immunofluorescence experiments show that the dosage compensation complex, responsible for balancing gene products from the two X chromosomes in hermaphrodites, is activated earlier in embryogenesis in the absence of *xol-1*. We also show that *xol-1* mutant embryos significantly overexpress *met-2*, which is a histone methyltransferase responsible for depositing the H3K9me2 mark. Overexpression of *met-2* and subsequent changes in H3K9me2 can provide an explanation for the differential embryonic developmental plasticity seen in our *xol-1* mutants.

1256T **GPCR Signaling, ACY-4, and acto-myosin contractility in *Caenorhabditis elegans* spermatheca** Maria Khalid[1], Erin Cram[2] [1]Biology, Northeastern university, [2]Biology, Northeastern University

Correct regulation of smooth muscle cell Ca^{2+} release and contractility are vital for the function of various biological systems, including the lungs and cardiovascular system. In the hermaphroditic nematode *Caenorhabditis elegans,* the reproductive system contains a contractile tube composed of smooth muscle-like cells called the spermatheca which stores sperm and is the site of oocyte fertilization. Regulated contractions of the spermatheca push the embryo into the uterus. Previous data suggest that GPCR signaling through the Gα subunits (GOA-1 and GSA-1), presumably upstream of adenylyl cyclase, plays a key role in regulating Ca^{2+} release in the spermatheca. Here, we describe the role of ACY-4/ADCY6, an enzyme that converts ATP into cAMP. ACY-4 is expressed in the spermatheca, particularly in the entry neck, and is required for the ovulation of oocytes into the spermatheca. Loss of function alleles of *acy-4* is characterized by stacked oocytes, endomitotic reduplication, eggs pushed back into the gonad arm, and infertility. Additionally, we demonstrate that *acy-4* depletion impacts the actin cytoskeletal organization in the spermatheca. These results suggest ACY-4 may act downstream of GPCR signaling in the spermatheca to regulate acto-myosin contractility.

1257T **ATPase Function of SMC proteins in Chromosome-wide Gene Regulation** Bahaar Chawla, Suchi Jatia, Dillon Sloan, Gyorgyi Csankovszki Molecular, Cellular, and Developmental Biology, University of Michigan

Structural Maintenance of Chromosome (SMC) proteins function in condensin complexes, hydrolyzing ATP for energy to condense DNA for mitosis. However, their role in interphase gene regulation is not well understood because most mutations in SMC proteins are lethal. *C. elegans* present a unique opportunity to study SMC proteins through their third condensin complex, condensin I^{DC}, which has a unique SMC protein DPY-27 that works specifically in the dosage compensation complex (DCC) to regulate X-chromosome gene expression. However, the contribution of the ATPase activity by condensin I^{DC} in this chromosomal-wide gene regulation is unknown.

We aim to understand this role with ATPase deficient *dpy-27* mutant worms and evaluating the effect on X chromosome structure, gene regulation, and other DCC functions. These studies will help us understand if condensin I^{DC} is a true motor like other condensins or if it only serves as a scaffold for DCC members.

DPY-27, like other eukaryotic SMC proteins, functions in a dimer with another SMC protein, MIX-1. *In vitro* biochemical analysis of DPY-27 and MIX-1 demonstrate that DPY-27 is a true ATPase. We mutated the essential glutamate in the Walker B motif to a glutamine and demonstrated *in vitro* that it disrupts ATPase function. We generated the same mutation in *C. elegans dpy-27* through CRISPR. Using this mutant, we found that the subunits of condensin I^{DC} localize with each other but result in two populations of condensin I^{DC}; one that is strongly DNA bound and the other is transiently. Further experiments have shown that the X chromosomes in mutant nuclei are significantly decondensed and mutant DPY-27 is not found exclusively on the X chromosomes.

Taken together, our results suggest that losing the ATPase function of condensin I^{DC} causes decreased binding of the DCC to the X chromosome, resulting in improper dosage compensation and a loss of viability.

1258T **Loss of the H3K4 methyltransferase subunit *wdr-5* in *C. elegans* results in a transgenerational decrease in chemotaxis** Mackenzie S Roberson[1], Jaely Z Chavez[1], Monica Reeves[2], Karen L Schmeichel[3], David J Katz[2] [1]Biology, Oglethorpe University, [2]Emory University, [3]Oglethorpe University

In *C. elegans* we have previously showed that mutations in *wdr-5*, an essential subunit of the COMPASS complex that is required for depositing H3K4 methylation, lead to the transgenerational enrichment of the repressive mark H3K9me2. To determine whether the accumulation of H3K9me2 affects behavior, we performed chemotaxis assays on *wdr-5* mutants. We find that in comparison to wild-type (N2), *C. elegans* mutant for *wdr-5* exhibit significantly reduced chemotactic behavior towards bacteria. Consistent with this being an inherited epigenetic effect, the chemotaxis index (C.I.) in *wdr-5* worms declines by 50.2% between the F4 and F7 generations ($C.I._{F4}$=0.80 and $C.I._{F7}$= 0.40). To our knowledge, this is the first example of a transgenerational behavior phenotype potentially caused by the accumulation of histone methylation cross generations. Interestingly, after the 7th generation, *wdr-5* mutants gradually restore their chemotactic behaviors to levels comparable to early generations ($C.I._{F13}$= 0.74). The transgenerational decline in chemotactic behavior and the subsequent reversion of phenotype is phenocopied in worms that lack the H3K9me2 demethylase *jhdm-1*. Collectively, these data are consistent with the interpretation that transgenerational establishment of repressed chromatin through the accumulation of H3K9me2 can result in abnormal behavior *C. elegans*. We are currently performing RNA sequencing and analyzing H3K9me2 Chromatin immunoprecipitation (ChIP) data to test this hypothesis.

1259T **H4K20 Methylation Regulation in Dosage Compensation and Cell Cycle of *C. elegans*** Anati Alyaa Azhar, Jianhao Jiang, Györgyi Csankovszki MCDB, University of Michigan, Ann Arbor

In *C. elegans*, hermaphrodites have two X chromosomes while males have a single X chromosome, leading to unequal gene expression. Dosage compensation is a process that solves this issue by equalizing this difference in gene expression between the two sexes. In *C. elegans*, hermaphrodites (XX) downregulate their X chromosomes by half to equalize it to males (X) via the Dosage Compensation Complex (DCC) in somatic cells. The DCC consists of a 5-subunit Condensin I^{DC} complex as well as additional accessory proteins that cooperate to downregulate the gene expression of the X chromosomes. One of the accessory subunits of the DCC is DPY-21, a H4K20-specific demethylase that enriches for H4K20me1 on dosage compensated X chromosomes which is the same chromatin mark enriched on mitotic chromosomes. We aim to understand the coordination between H4K20me1 regulation in dosage compensation and the cell cycle.

In *C. elegans*, H4K20 methylation is affected by SET-1, a methyltransferase that places the first methyl mark on H4K20; SET-4, which converts H4K20me1 to di- and tri-methylation; and DPY-21, a non-condensin subunit of the DCC, which demethylates H4K20me2/3 to H4K20me1 on the X chromosomes. We show that in young embryos, where the cell cycle switches between M and S phases, DPY-21 and SET-4 play a limited role in the regulation of H4K20 methylation. However, in late embryos which have cell cycles with gap phases, the two proteins begin to affect H4K20 methylation. In dosage compensated wild-type late embryos, H4K20me1 decreases in all chromosomes in S phase, increases in all chromosomes during mitosis, and is enriched on hermaphrodite X chromosomes during G1 phase. However, H4K20me in G1 phase is disrupted in the mutants whereby H4K20me1 is diffusely distributed on all chromosomes in dpy-21 mutant and H4K20me1 is present in high levels on all chromosomes in set-4 mutant. We show a complementary trend with H4K20 di- and tri-methylation, in which H4K20me2/3 is present on all chromosomes in dpy-21 mutant while it is diminished on all chromosomes in set-4 mutant. Thus, SET-4 and DPY-21 have the greatest impact on H4K20me during G1. Our goal is to characterize the function and regulation of these two proteins by identifying their interactors and regulators.

1260T **Starvation changes the pre-rRNA accumulation in *C. elegans*** Shahriar Rahman Shovon[1], Naoki Uematsu[2], Yuki Osaki[2], Tatsushi Masui[2], Chisato Ushida[1,2] [1]Bioresources Science, Iwate University, [2]Biochemistry and Molecular Biology, Hirosaki University

The depletion of nutrients significantly impacts cellular processes across all organisms, triggering a restructuring of ribosome biogenesis. Ribophagy, a specialized process involving the selective degradation and recycling of pre-ribosomes and mature ribosomes during stress, is a pivotal response to nutrient depletion. However, the specific effects of nutrient scarcity on ribosome biogenesis remain elusive. Our investigations revealed a novel mechanism regarding the alteration in the accumulation pattern of two distinct ribosomal RNA (rRNA) precursors, namely rRNA precursor b (a precursor of 18S rRNA) and rRNA precursor $27SA_2$ (a precursor of 5.8S and 26S rRNAs), particularly under short-term starvation conditions. Interestingly, we observed a substantial decrease in the $27SA_2$ rRNA precursor, while there was a notable increase in the accumulation of b rRNA precursor. Intriguingly, the rRNA processing pathway recovered when starved animals were provided with an adequate food supply. This observation suggests a novel cellular mechanism for selectively degrading the $27SA_2$ precursor while potentially recycling nucleic acids to adapt to nutritional deprivation. Moreover, the precise cleavage site responsible

for dissociating the 5.8S rRNA precursors and 26S rRNA precursors in *C. elegans* remains unidentified. Through our northern hybridization experiments, we successfully identified the precursor of 5.8S rRNA. Subsequent primer extension experiments further confirmed the precise cleavage position at the internal transcribed spacer 2 (ITS2), which generates the precursor of 5.8S rRNA and the precursors of 26S rRNA. These findings underscore the intricate adaptations within ribosome biogenesis in response to short-term nutritional stress, shedding light on the selective degradation and potential recycling of specific rRNA precursors. Nevertheless, further investigations are imperative to fully elucidate the mechanisms governing this intricate cellular response to nutrient depletion.

1261T **Exploring Novel Intracellular Pathogen Response (IPR) Triggers in *Caenorhabditis elegans*: Analyzing the Impact of Ethanol on Intestinal Permeability and its implications for Innate Immunity in Humans** Iris Kazzi[1], Adrian Alvarado[2], Jessica Sowa[3] [1]Chemistry, West Chester University of Pennsylvania, [2]Biology, West Chester University of Pennsylvania ylvania, [3]Biology, West Chester University of Pennsylvania

The ability to develop and sustain immune responses in organisms plays an important role in their survival against pathogens and stressors. In the nematode *Caenorhabditis elegans*, the Intracellular Pathogen Response (IPR) functions as an important immune defense mechanism against a range of stressors and pathogenic threats. While viral infection, proteasome activity, and exposure to heat stress are recognized as triggers for activating the IPR in *C. elegans*, our research findings have unveiled a previously unrecognized inducer: Ethanol-induced intestinal wounding in *C. elegans*, leading to the upregulation of IPR-related genes. This discovery raises the hypothesis that intestinal wounding may represent a distinct branch within the IPR activation pathway but the factors responsible for initiating and defining this pathway and its implications for innate immunity in humans remain to be solved. Our research has identified 6% ethanol as a novel method for inducing intestinal wounding, thereby stimulating IPR activation. This discovery presents the potential for its application in high-throughput procedures, facilitating genome-wide screenings and advancing our understanding of this intricate immune response mechanism.

1262T **Targeted mutations of miRNA duplexes reveal asymmetries important for proper strand selection *in vivo*** Jeffrey C Medley[1], Sumire Kurosu[1], Ganesh Panzade[2], Sarah Coffey[1], Will Sydzyik[1], Joel Sydzyik[1], Mira Bhandari[3], Anna Zinovyeva[1] [1]Division of Biology, Kansas State University, [2]Frederick National Laboratory for Cancer Research, [3]Department of Molecular and Integrative Physiology, University of Michigan

Animal development relies on timely expression and inactivation of genes to achieve diverse cellular functions. microRNAs (miRNAs) are non-coding RNAs that play a central role in the regulation of eukaryotic gene expression, typically by repressing their target genes. miRNAs are genomically encoded as double-stranded molecules that are processed into a duplex comprising two functionally distinct strands. One dominant (guide) strand is loaded into an Argonaute (Ago) protein to form the miRNA-induced silencing complex (miRISC), while the other (passenger) strand is degraded. As miRNAs target genes based on nucleotide base-pairing, the asymmetric decision of which strand is loaded into Ago effectively determines the target repertoire of miRISC. Previous studies have suggested that 5' nucleotide identity and thermodynamic asymmetry of miRNA duplex ends is sufficient to explain which strand is preferred for Ago loading *in vitro* and can predict which strand is selected *in vivo*. However, many mature miRNAs do not have sequence features favorable for Ago loading. In addition, the actual ratio of each strand remains difficult to predict. Here, we tested different approaches for calculating duplex end stabilities and assessed how each approach predicted experimentally observed *in vivo C. elegans* miRNA strand ratios. By testing different models of thermodynamic asymmetry, we were able to closely predict the direction and relative strand ratios of most *C. elegans* miRNAs. We find that strand predictions can sometimes be improved by considering how miRNA precursors may constrain the duplex structure, rather than only considering the minimum free energy state of the duplex. To test different strand selection models, we mutated three endogenous *C. elegans* miRNAs to alter their duplex characteristics and examine how strand selection was affected. We found mutations affecting both 5' nucleotide and thermodynamic asymmetry were sufficient to reverse strand selection of each miRNA *in vivo*, although the relative contribution of each factor appeared miRNA-dependent. In part, these differences might be explained by differences in the relative stability of each miRNA strand, or inability of certain strands to stably load into Ago. Current work is aimed at refining strand selection predictions and examining the contributions of Agos and other factors towards proper strand selection. Collectively, our findings provide insights into how asymmetric miRNA strand selection is achieved *in vivo*.

1263T **Cohesin mediated loop extrusion from active enhancers form chromatin jets in *C. elegans*** Sevinc Ercan, Jun Kim New York University

In mammals, cohesin and CTCF organize the 3D genome into topologically associated domains (TADs) to regulate communication between cis-regulatory elements. However, many organisms, including S. cerevisiae, C. elegans, and A. thaliana lack CTCF. Here, we use C. elegans as a model to investigate the function of cohesin in 3D genome organization in an animal without CTCF. We use auxin-inducible degradation to acutely deplete SMC-3 or its negative regulator WAPL-1 from

somatic cells. Using Hi-C data, we identify a cohesin-dependent 3D genome feature termed chromatin jets (aka fountains), also observed in zebrafish and mammalian genomes. The jets emerge from NIPBL occupied segments, and the trajectory of the jets coincides with cohesin binding. The spreading of cohesin from jet origins depends on a fully intact cohesin complex and is extended upon WAPL-1 depletion. These results support the idea that cohesin is preferentially loaded at NIPBL occupied sites, from which cohesin loop extrudes in an effectively two-sided manner. The location of putative loading sites coincides with active enhancers and the pattern of chromatin jets correlates with transcription. We propose that in the absence of CTCF, preferential loading of cohesin at enhancers is a conserved mechanism of genome organization that regulates the interaction of gene regulatory elements in 3D.

1264T **S-adenosylmethionine synthases specify distinct H3K4me3 populations and gene expression patterns during heat stress** Amy K Walker[1], Adwait Godbole[2], Alexander Munden[2] [1]Program in Molecular Medicine, UMASS Medical School, [2]PMM, UMASS Medical School

Methylation is a widely occurring modification that requires the methyl donor S-adenosylmethionine (SAM) and acts in regulation of gene expression and other processes. SAM is synthesized from methionine, which is imported or generated through the 1-carbon cycle (1CC). Alterations in 1CC function have clear effects on lifespan and stress responses, but the wide distribution of this modification has made identification of specific mechanistic links difficult. We hypothesize that provisioning of SAM through specific synthases provides a level of regulatory specificity to methylation dependent pathways. In most animals, SAM may be synthesized by one of several SAM synthases. For example, mammals contain two SAM synthases, one of which is specific to adult liver (MAT1A). While MAT2A is ubiquitous, it may exist in multiple isoforms. The SAM synthase family in C. elegans is expanded, with 4 isoforms. Sams-1 is the most highly studied, with roles in lifespan extension, lipid storage and complex function in the stress response. Lowering SAM to similar levels by limiting *sams-4* is not sufficient to recapitulate these phenotypes, suggesting it is not the level of SAM, but how or where it is produced that provides the regulatory specificity. Tissue specific effects could explain some functional differences. However, using CRISPR tagged alleles, we find that SAMS-1 and SAMS-4 are largely co-expressed with the exception of the germline, which lacks SAMS-1.

We are leveraging the heat stress response to identify regulatory effects controlling differential effects. Reduction in these enzymes produces opposite phenotypes, distinct heat responsive gene expression programs, metabolic changes and H3K4me3 methylation patterns. We also find that SAMS-4 initiates a distinct histone methylation program in the absence of SAMS-1 that impacts survival. Our preliminary data suggest SAMS-1 and SAMS-4 have some distinctions in their local proteomes, suggesting potential for distinct methylation targets or regulatory interactions. Taken together, our results suggest that the regulatory functions of SAM depend on its enzymatic source and that provisioning of SAM may be an important regulatory step linking 1CC function to phenotypes in aging and stress.

1265T **Optimization of CUT&RUN for lowly expressed transcription factors in C. elegans' larval stages** Jada Coffey, Dagmawi Lulseged, Alexis Roudenko, Deborah M Thurtle-Schmidt Biology, Davidson College

Genome-wide chromatin profiling is a vital technique that elucidates interaction between transcription factors and DNA. These interactions are necessary for proper gene regulation. Cleavage Under Targets and Release Using Nuclease (CUT&RUN) is a recently developed chromatin profiling method that allows for profiling specific DNA-protein interactions in a short amount of time and with much less background noise. CUT&RUN uses antibody-targeted cleavage to release specific protein-DNA fragment complexes for DNA sequencing. Chromatin profiling techniques have been difficult to adapt to *C. elegans* due to the worm's tough cuticle. Two studies thus far have adapted CUT&RUN for *C. elegans*, using abundant histone epitopes or in adults. Here we optimize the CUT&RUN protocol for *C. elegans* to characterize lowly expressed transcription factors at larval stages. We optimized our worm-growing and bleaching protocol, to isolate a sufficient number of worms at the correct larval stage. *C. elegans* were permeabilized using a grinding protocol and Tapestation and Qubit analysis indicated DNA recovery. The sequencing analysis is pending. In future studies, we will adapt this CUT&RUN protocol to determine if EOR-1 is a pioneer factor for NHR-25 through profiling of NHR-25 binding in the presence and absence of *eor-1*.

1266T **Prophage proteins modulate eukaryotic long non-coding RNA and DNA to spread *Wolbachia* symbiont** Rupinder Kaur[1], Angelina McGarry[1], Dylan J Shropshire[2,3], Brittany A Leigh[2], Isabella T Ritchie[2], Seth R Bordenstein[4] [1]Biology, Pennsylvania State University, [2]Vanderbilt University, [3]Lehigh University, [4]Pennsylvania State University

The widespread distribution of endosymbiotic bacteria *Wolbachia* in arthropods and massive success in mosquito control efforts is in part due to a drive system named cytoplasmic incompatibility (CI). CI confers a paternal-effect, embryonic lethality when sperm from *Wolbachia*-bearing testes fertilize eggs from uninfected females. The rising success in deploying CI for vector control strategies necessitates an explanation of the CI mechanism. In *Drosophila melanogaster* carrying *w*Mel *Wolbachia* strain, CI is induced by two prophage-WO encoded proteins – CifA and CifB that

target sperm nuclei during development. Here we characterize the cellular and biochemical properties of the Cif proteins and show that 1) CifA is a RNase enzyme that diminishes a spermatocyte-expressing long non-coding (lnc) RNA earlier in spermatogenesis, and knockdown of the lncRNA in spermatocytes enhances CI levels, 2) CifA and CifB are both DNases that elevate DNA damage in elongating spermatids, 3) Cifs alter the sperm chromatin integrity by impairing histone and protamine nucleoproteins composition during late spermiogenesis, and finally 4) the uninfected embryo fertilized by compromised CI sperm suffers from DNA damage during nuclear divisions leading to embryonic death. We conclude that the mechanism of CI-induced embryonic death relies on a cascade of paternal chromatin integrity changes pre-established in testes during sperm development. This work unlocks a previously-unrecognized phenomenon by which prophage proteins alter central dogma features of *Drosophila* gametogenesis to fashion a worldwide symbiosis with relevance to vector control and insect evolution.

1267T **Targeting an Active Chromatin Domain to the Drosophila X-chromosome** Melissa Aldana, Angelica Aragon Vasquez, Claire Gray, Joseph Aguilera, Mukulika Ray, Erica Larschan MCB, Brown University

In male Drosophila, the Dosage Compensation Complex (DCC) is targeted to the X-chromosome via a transcription factor-Chromatin-linked Adaptor for MSL complex (CLAMP). Targeting the DCC to the X-chromosome upregulates the active genes along the single X-chromosome in males two-fold, equalizing the X-chromosome transcriptional output with that from the two autosomes. Different regions of CLAMP were excised to dissect the regions necessary for proper DCC distribution along the X-chromosome. However, CLAMP binds to all chromosomes and therefore the key question is: How does CLAMP specifically target the DCC to all chromosomes? I performed Cleavage Under Targets and Release Using Nuclease (CUT&RUN) to better understand DCC targeting the X-chromosome in CLAMP mutants. After isolating the DNA fragments post-CUT&RUN, I prepared for Next Generation Sequencing. I obtained my sequencing data, I performed analyses to investigate DCC binding with Deeptools. Looking at the conserved motifs in this region, high promiscuity suggests other transcription factors can bind at the sites where there are not as many conserved motifs. Using ReactomePA, the pathway and gene ontology enrichment clusters in clamp mutants but not wild type are significantly associated with programmed cell death suggesting that CLAMP prevents binding to genes that induce cell death.

1268T **The single-cell transcriptome and chromatin landscape analysis elucidates synaptic gene regulation during Drosophila embryonic development** Tuan Pham, Justin Currie, James Kentro, Gunjan Singh, Erica Larschan, Kate O›Connor-Giles Brown University

Coordinated gene regulation is essential for the generation and maintenance of diverse cellular states during development. The precise activation or repression of transcription is primarily controlled by transcription factors (TFs) that form gene regulatory networks (GRNs) amongst themselves and with their target genes.The Drosophila brain is an excellent model in neuroscience. Leveraging an atlas of scRNA-seq and scATAC-seq from the Drosophila embryonic development and current computational methods, we have identified specific GRNs modulating synaptic genes for 6 timepoints during nervous system development from 6h to18h. Our findings not only show well-defined regulators of synaptic genes consistent with current literature but also less-studied TFs that are excellent candidates for further experimental investigation in nervous system development. We also highlight noticeable similarities and changes over time in GRNs that might be key to understanding temporal regulation of the brain.

1269T **Impacts of Heat Shock on Transcriptional Noise Driven by Developmental Shadow Enhancers** Julia Gibbs[1], Zeba Wunderlich[2], Jillian Ness[2] [1]Biology, Boston University, [2]Boston University

The precise control of developmental gene expression drives the development of the whole organism, from specifying individual cells to patterning tissue development. Yet this occurs through inherently noisy processes, like transcription and translation, and often does so under stressful environmental conditions. Stress can increase noise in a system, as it disrupts several processes central to normal cell activity, and no species can escape heat waves, infection, or other stresses that can occur during development. Thus, several control mechanisms have evolved to control noise in developmental processes, even under stressful conditions. One of these mechanisms is shadow enhancers, sets of two or more enhancers that control the same gene and drive overlapping gene expression patterns. These enhancer sets are often not necessary for proper development under ideal conditions. However, under stressful conditions, they drive developmental stability, lowering noise in developmental gene expression and promoting formation of wild-type flies. How exactly they achieve this stability, and this lowering of noise, still needs to be explored. Previous work in the lab measured the expression dynamics of the Kruppel shadow enhancer system in early Drosophila embryos and found that the individual enhancers in the set have distinct reactions to heat stress. While the promoter-distal enhancer of the system shows the expected increase in transcriptional noise under stress, the promoter-proximal enhancer showed no significant changes in noise. There are several possible explanations for this, but central amongst them is the structure of the enhancer itself, i.e. its transcription factor binding sites and the histone marks present in vivo. To gain insight into distinct reactions of enhancers to heat stress, I performed ATAC-Seq on embryos at ideal and elevated temperatures and analyzed the resulting data using the TF footprinting method TOBIAS. This

allowed me to gather data on both chromatin accessibility changes and the changes in TF binding that occur under heat shock. Using this data, I will investigate how TF binding and histone marks affect an enhancer's response. I will use ChIP-Seq to more directly probe TF binding mechanics, or histone modifications, that affect each individual enhancer, and combine that with my ATAC-Seq data to understand each enhancer's stress reaction. From this, we will better understand how developmental genes maintain robust expression, and potentially, how we can recapitulate that robustness when developmental systems begin to fail.

1270T **Characterization of Bedwarfed in genome organization and transcription in *Drosophila*** Dagyeong Yang[1,2], Elissa P. Lei[1] [1]NIDDK/NIH, [2]University of Maryland College Park

Three-dimensional (3D) spatial organization of the interphase genome is an important factor influencing gene regulation. The interphase genome segregates into highly self-interacting topologically associated domains (TADs) that influence regulation of gene expression. The boundaries of TADs are maintained in part by DNA-protein complexes termed chromatin insulators, including the universal Centrosomal Protein 190kD (Cp190). In addition to chromatin insulators, these boundaries are often marked by active transcription and enrichment of the core promoter, motif-1. Recent studies have shown that a transcription factor that binds to motif-1, M1BP, and CP190 physically interact and are together required for motif-1 dependent gene expression and transcription near TAD boundaries.

Along with M1BP, an *in vitro* pull-down assay identified several motif-1 associated proteins, including Gfzf, Putzig, and Bdwf (Bedwarfed). Bdwf has been shown to function in *Drosophila* neuronal development. However, a functional relationship between Bdwf and motif-1 sequence remains unknown. In this study, we analyze the potential function of Bdwf in genome organization and gene regulation. Our analysis of ChIP-seq data from the ENCODE project revealed substantial overlap of Bdwf with CP190, M1BP, and BEAF-32 at TAD borders. Utilizing MEME-ChIP, we confirmed the enrichment of motif-1 at Bdwf binding sites throughout the genome. Ongoing research aims to elucidate how Bdwf contributes to motif-1 dependent gene expression using a plasmid-based luciferase reporter assay. Moreover, RNAseq analysis revealed enrichment of differentially expressed genes near TAD borders after depletion of Bdwf in Kc cells, suggesting a role for Bdwf in regulating gene expression at these sites. To further understand its role in transcription, we will perform Nascent RNAseq. Overall, our studies will reveal the function of Bdwf in *Drosophila* genome organization and transcriptional regulation.

1271T **Genome organization mediated by a MADF-BESS protein is critical for nuclear pore complex production and cell fate transition during Drosophila oogenesis** Noor M Kotb[1], Gulay Ulukaya[2], Ankita Chavan[3,4], Lydia Proskauer[5], Eric Joyce[6], Dan Hasson[2], Madhav Jagannathan[7], Prashanth Rangan[8] [1]CDRB/BMS, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA/ University of Albany, Albany,NY 12207,USA, [2]Tisch Cancer Institute Bioinformatics for Next Generation Sequencing (BiNGS) core, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA, [3]Department of Biology, Institute of Biochemistry, ETH Zürich, 8092 Zurich, [4]Department of Biology, Institute of Biochemistry, ETH Zürich, [5]Biochemistry and Molecular Biology Department, College of Natural Sciences, University of Massachusetts Amherst, [6]Department of Genetics, University of Pennsylvania, Philadelphia, PA 19104, [7]Department of Biology, ETH Zürich, 8092 Zurich, [8]Department of Cell, Developmental, and Regenerative Biology, Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

Genome organization can regulate gene expression and promote cell fate transitions. The differentiation of germline stem cells (GSCs) to oocytes in Drosophila melanogaster involves changes in genome organization mediated by heterochromatin and the nuclear pore complex (NPC). Heterochromatin represses germ-cell genes during differentiation and NPCs anchor these silenced genes to the nuclear periphery, maintaining silencing to allow for oocyte development. Surprisingly, we find that genome organization also contributes to NPC formation, mediated by the transcription factor Stonewall (Stwl). As GSCs differentiate, Stwl accumulates at boundaries between silenced and active gene compartments. Stwl at these boundaries plays a pivotal role in transitioning germ-cell genes into a silenced state and activating a group of oocyte genes and Nucleoporins (Nup) by regulating enhancer activity. The upregulation of these Nups during differentiation is crucial for NPC formation and further genome organization. Thus, crosstalk between genome architecture and NPCs is essential for successful cell fate transitions.

1272T **KDM5-mediated transcriptional activation of ribosomal protein genes alters translation efficiency to regulate mitochondrial metabolism in neurons** Matanel Yheskel[1], Hayden AM Hatch[2], Erika Pedrosa[3], Bethany K Terry[1], Xiang Yu[1], Laura E.R. Blok[4], Michaela Fencková[5], Simone Sidoli[6], Annette Schenck[4], Deyou Zheng[1], Herbert M Lachman[7], Julie Secombe[8] [1]Genetics, Albert Einstein College of Medicine, [2]Neuroscience, Albert Einstein College of Medicine, [3]Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine, [4]Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, [5]Molecular Biology and Genetics, University of South Bohemia, [6]Biochemistry, Albert Einstein College of Medicine, [7]Medicine, Albert Einstein College of Medicine, [8]Albert Einstein College of Medicine

Genes encoding the KDM5 family of transcriptional regulators are disrupted in individuals with intellectual disability (ID). To understand the link between KDM5 and ID, we characterized five *Drosophila* strains harboring missense alleles analogous to those observed in patients. These alleles disrupted neuroanatomical development, cognition and other behaviors, and displayed a transcriptional signature characterized by the downregulation of many ribosomal protein genes. A similar transcriptional profile was observed in *KDM5C* knockout iPSC-induced human glutamatergic neurons, suggesting an evolutionarily conserved role for KDM5 proteins in regulating this class of genes. In *Drosophila*, reducing KDM5 changed neuronal ribosome composition, lowered the translation efficiency of mRNAs required for mitochondrial function, and altered mitochondrial metabolism. These data highlight the cellular consequences of altered KDM5-regulated transcriptional programs that could contribute to cognitive and behavioral phenotypes. Moreover, they suggest that KDM5 may be part of a broader network of proteins that influence cognition by regulating protein synthesis.

1273T **Role of different protein domains in context dependent function of transcription factors** Victoria Chen, Mukulika Ray, Jasmine Shum, Pranav Mahableshwarkar, Julia Zaborowsky, Nicolas Fawzi, Erica Larschan Brown University

Transcription factors (TFs) play an important role in regulating spatiotemporal gene expression. It remains unclear how the same TF controls multiple processes and is targeted to the right genomic location in the right cellular context. In the present study, we explore the role of different protein domains in deciding TF function. We use the GA-rich DNA binding transcription factor CLAMP (Chromatin-linked adapter for MSL proteins) as a model.

CLAMP is enriched on the Male-X chromosome and helps in recruitment of the Male-sex lethal complex (MSL complex) that increases transcription on the Male-X chromosome, a process called dosage compensation since it maintains the ratio of transcription between XX female and XY male individuals. Also, CLAMP is important for sex-specific splicing in female and male individuals from early embryonic stage. We studied the role of different regions of the CLAMP protein in regulating both of these functions.

The N-terminal 52-153 amino acid (a.a) region with a zinc finger domain in the CLAMP protein is important for binding to the MSL complex protein MSL2. Adjacent to the MSL2 binding domain is the disordered glycine-rich PrLD region (153-292 a.a), known to be important for RNA binding. Earlier studies in CRISPR-generated fly mutants have shown that deletion of the N-terminal Zinc Finger domain and Prion-like domain (PrLD) results in CLAMP-dependent splicing changes. The former mutant affected distribution of MSL complex protein MSL2. We hypothesize that different domains of CLAMP influence CLAMP interaction with specific DNA and RNA sequences, thus resulting in specific function.

In the lab, we had already expressed PrLD-deleted CLAMP proteins in the bacterial expression system with the help of the Fawzi Lab. We also used PCR-mutagenesis to generate plasmids with deletions in the Zinc Finger (ZnF) domain of the full-length and 1-300 amino acid long CLAMP. Mutant proteins using these plasmids were expressed, which were used to perform RNA and DNA gel shift assays to determine protein-nucleic acid interactions. We found that the PrLD domain of CLAMP is essential for binding to specific RNA sequences. We also identified specific RNA sequences that aid in binding to CLAMP proteins. Furthermore, full-length CLAMP has better binding efficiency than 1-300 length CLAMP.

1274T **SUMO inhibits Scm polymerization and association with developmentally-silenced target genes** Steven Z DeLuca[1,2], Tian Tan[2], David Lyons[1] [1]Microbiology and Cell Biology, Montana State University, [2]Biology, Brandeis University

During animal development, Polycomb-group (PcG) proteins associate with tissue-specific target genes to inhibit their inappropriate expression in the wrong tissues. In the correct tissue, Trithorax-group (TxG) proteins associate with target genes to somehow prevent PcG proteins from inhibiting them. There are likely multiple mechanisms through which TxG proteins inhibit PcG proteins. Here, we use the Drosophila ovary model system to identify a new TxG protein, Su(var)2-10, that is important for preventing the silencing of the expressed target gene, caudal. Furthermore, we show that Su(var)2-10 promotes the SUMOylation of the PcG protein, Scm, on three lysine residues. These SUMOylated lysines allosterically inhibit Scm from polymerizing and fully associating with Polycomb Response Elements (PREs) on target genes like caudal. Through genetic alterations to the Scm polymerization domain and SUMOylation sites, we show that polymerization correlates with PRE occupancy and target gene silencing. Together, our experiments suggest a new model where Scm is differentially SUMOylated and polymerized on target genes to regulate their expression or silencing.

1275T **Histone H3 lysine 4 is required for proper activation of Polycomb target genes** Cyril S Anyetei-Anum[1], Daniel J. McKay[2,3,4] [1]Curriculum in Genetics & Molecular Biology, University of North Carolina Chapel Hill, [2]Department of Biology, University of North Carolina Chapel Hill, [3]Department of Genetics, University of North Carolina Chapel Hill, [4]Integrative Program for Biological and Genome Sciences, University of North Carolina Chapel Hill

Polycomb Group (PcG) genes encode a highly conserved group of epigenetic regulators required for maintenance of cell

identities. They function through heritable silencing of important developmental genes such as the Hox genes. There are two major Polycomb Repressive complexes, PRC1 and PRC2, that have different roles in target gene repression. PRC2 contains the histone methyltransferase subunit E(z) in *Drosophila* that trimethylates histone H3 lysine 27 (H3K27me3). PRC1 binds this post-translational modification (PTM), and together with PRC2, forms repressive chromatin domains that extend across silenced target genes. Trithorax Group (TrxG) proteins, by contrast, are required to promote gene expression. The TrxG founding member, Trithorax, is a methyltransferase specific to histone H3 lysine 4 (H3K4) -- a PTM tightly associated with active genes. Genetic studies indicate that Trx antagonizes the function of PcG complexes, leading to current models in which the balance of Trx and PcG activity determines whether target genes are expressed or repressed. However, the mechanisms underlying this antagonism are unclear. One line of evidence suggests a contribution of H3K4 methylation in controlling the balance between the on and off state. Namely, H3K4me3 peptides inhibit the enzymatic activity of PRC2 *in vitro*. Here, we test the functional role of H3K4 in the balance between the active and repressed states *in vivo*. We employ a genetic platform to replace the endogenous replication-dependent histone genes with transgenic versions engineered to encode a non-modifiable H3.2K4 residue (H3.2K4R). We find that H3.2K4R mutants die early in development with decreased H3K4 methylation levels. Surprisingly, H3.2K4R mutants also exhibit decreased H3K27me3 levels, suggesting a defect in PcG function. However, despite lower H3K27me3 levels, H3.2K4R mutant cells do not exhibit Hox gene derepression, suggesting that H3.2K4 contributes to PcG target gene activation. To directly test whether H3.2K4 is required for activation of PcG target genes, we generated replacement flies expressing H3.2K4R,K27R double mutant histones. We find that Hox gene derepression is significantly reduced relative to levels observed in H3.2K27R single mutant cells. Collectively, our studies support a role for H3.2K4 in PcG target gene activation.

1276T **Transcriptomic analyses of Set2 and H3^{K36R} mutants reveal heterogeneous regulation of X chromosome gene expression** Harmony Salzler[1], Vasudha Vandadi[1], A. Gregory Matera[1,2,2,3,4] [1]Integrative Program for Biological and Genome Sciences, UNC Chapel Hill, [2]Genetics, UNC Chapel Hill, [3]Biology, UNC Chapel Hill, [4]Lineberger Comprehensive Cancer Center, UNC Chapel Hill

Different sex chromosome karyotypes present a challenge to balanced X chromosome gene expression in many species. In flies, dosage compensation (DC) is achieved by upregulation of male X genes by the male-specific lethal (MSL) complex, which binds high-affinity sites and spreads to surrounding genes, propagating H4 lysine 16 (H4K16) acetylation. A full understanding of MSL complex spreading has been elusive, but current models focus on the potential of male-specific lethal 3 to interact with modified histone tails, in particular Set2-dependent trimethylation of H3 lysine 36 (H3K36me3). Recent work in the DC field questions the relevance of H3K36me3 to MSL spreading, suggesting that Set2 may exert an effect on DC via another target. While intriguing, the possibility of redundancy between lysine 36 of H3.2 and H3.3 histone variants has not been sufficiently investigated, and distinctions between canonical (MSL mediated) DC and other modes of X chromosome regulation (applicable to females) have not been explored.

To address these questions, we employed genomic approaches in *H3.2^{K36R}*, *H3.3^{K36R}*, *H4^{K16R}*, and *Set2Δ* mutants. Larval brain RNA-Seq in both sexes confirms a role for Set2 in X chromosome gene regulation and reveals that many genes bound by high levels of MSL complex in males follow the same gene expression trends in females (where the MSL complex is inactive), albeit to a lesser degree. Furthermore, *k*-means clustering of expression changes uncovers surprisingly heterogeneous effects of Set2 and H3K36 on X chromosome genes. Intriguingly, we observe gene groups where residue and writer mutations push gene expression in opposite directions, suggesting that other modification states of H3K36 may be important regulators of these genes. To address redundancy between H3 variants, we performed RNA-Seq in L1 animals combining *H3.2^{K36R}* and *H3.3^{K36R}* mutations. These data suggest that H3.2K36 and H3.3K36 are largely redundant in regulation of X genes. Importantly, heterogeneous expression changes in *Set2Δ* and *H3^{K36R}* mutants contrast strongly to the ubiquitous reduction in X chromosome gene expression in *H4^{K16R}* mutants. We also observe that *Set2* mutation exerts opposite effects on many genes at this different tissue/stage, suggesting significant context dependence. Overall, these data are inconsistent with the prevailing model where Set2 and H3K36 directly recruit the MSL complex. Rather we hypothesize that Set2 via H3K36 supports DC indirectly by regulating processes involved in DC, but common to both sexes.

1277T **Defining Activities of KDM5 Essential to Development and Viability** Melissa Castiglione[1], Hayden Hatch[2], Andreas Bergmann[3,4], Hans Martin-Herz[4,5], Julie Secombe[2,6] [1]Albert Einstein College of Medicine, [2]Neuroscience, Albert Einstein College of Medicine, [3]University of Massachusetts Chan Medical School, [4]MD Anderson Cancer Center, [5]St. Jude Children's Research Hospital, [6]Genetics, Albert Einstein College of Medicine

The Lysine demethylase 5 (KDM5) family of transcriptional regulators are important for normal development, and their dysregulation is a key driver of intellectual disability and several forms of cancer. In this work, we examine essential functions of KDM5 via a novel truncation allele, *kdm5Q19. kdm5Q19* inserts a stop codon in a previously unrecognized, evolutionarily conserved, motif within an intrinsically disordered region of KDM5 at the C-terminus, removing the final 311 amino

acids. *kdm5Q19* animals do not survive to adulthood and have a slight developmental delay, a phenotype distinct from null, demethylase dead, and other mutants generated in our lab, suggesting that the region disrupted by the truncation has an essential as-yet-unknown role in normal KDM5 function in development. To further dissect the molecular activities of this region of KDM5, we generate additional alleles of *kdm5* to refine the critical region(s) of the protein and assess viability and changes to transcription.

In this work, we will (1) define essential regions within KDM5 required for viability, and to characterize their roles in development (2) define the essential molecular functions imparted by the C-terminus of KDM5. Together, these studies will refine the critical region(s) of the C-terminus of KDM5, and define the normal role of these regions in regulating transcription.

1278T **Determining essential, pioneering features of the conserved transcription factor Grainy head** Meghan M Freund[1], Tyler J Gibson[1], Andrew Q Rashoff[1], F. Javier deHaro-Arbano[2], Sarah Baloul[2], Abby J. Ruffridge[1], Peter W. Lewis[1], Sara J. Bray[2], Melissa M. Harrison[1] [1]University of Wisconsin-Madison, [2]University of Cambridge

Beginning from a single cell, organisms undergo a journey of differentiation that results in many unique cell types. Because these cells all possess the same genome, distinct cell types do not arise due to differences in genotype. Instead, regulation of gene expression drives cell-type specification. Transcription factors play an important role in this process by binding DNA to promote gene expression. Nonetheless, compacted chromatin acts as a barrier to transcription-factor binding. A specialized class of transcription factors, known as pioneer factors, overcome this barrier by binding to condensed chromatin and increasing chromatin accessibility. The mechanisms governing pioneer factor activity are not well understood. Grainy head (Grh) is an essential and highly conserved pioneer factor that drives epithelial cell fate and when mis-expressed leads to cancer. We use Grh as a model to explore the fundamental mechanisms by which pioneer factors engage the genome and shape cellular identity. A combination of biochemistry, genomics, and quantitative imaging has begun to elucidate key features of Grh. We showed that full-length Grh binds nucleosomes *in vitro* and drives accessibility when exogenously expressed in tissue culture. This pioneering activity requires DNA binding, but the DBD is not sufficient. Quantitative imaging revealed that Grh stably occupies the genome, likely an essential feature of pioneer activity, and that regions outside the DBD are required for stable occupancy. Altogether this suggests that regions within and outside the Grh DBD are instrumental in pioneering. Additionally, expression of mammalian orthologs in *Drosophila* tissue culture demonstrates that pioneering is a conserved feature of Grh. A subset of pioneer factors, including Grh, is retained on compacted mitotic chromosomes, yet the relationship between mitotic retention and pioneering function remains unclear. Residues that mediate DNA binding are necessary for mitotic retention, but regions outside the DBD are dispensable. This contrasts with the requirement of additional domains for stable-genome occupancy and pioneer-factor function. By exploring the properties of Grh using multiple, complementary assays, we provide fundamental insights into the mechanisms by which a conserved pioneer factor engages the genome to promote chromatin accessibility and drive cell fate.

1279T **Interactions Between the Histone Variant H2Av and Insulators in *Drosophila* Influence the DNA Damage Response and Lymph Gland Development** James R Simmons[1], Justin D. J. Kemp[2], Mariano Labrador[3] [1]Biochemistry and Cell and Molecular Biology, The University of Tennessee at Knoxville, [2]Biochemistry and Cell and Molecular Biology, The University of Tennesse, [3]University of Chicago Alumni

Genome architecture is regulated by chromatin insulator proteins, and misregulation of insulator function is associated with genome instability and transcriptional regulatory defects in both vertebrates and *Drosophila*. Indeed, mutations of the sole insulator protein in humans, CTCF, are carcinogenic and mutations in the *Drosophila* insulator protein *Suppressor of Hairy wing* [Su(Hw)] lead to chromosomal rearrangements. However, the mechanism that links the DNA damage response and the regulation of transcription with insulator function is not yet understood. Here we show that enrichment of Su(Hw) insulator proteins at insulator sites increases after DNA damage. Additionally, Su(Hw) is necessary for phosphorylation of the histone variant H2Av in response to both UV treatment and X-ray irradiation. Similarly, we provide evidence that Su(Hw) and H2Av work together to ensure proper development of the lymph gland in *Drosophila* larvae. We show that H2Av regulates formation of the larval lymph gland, and mutation of H2Av causes formation of large melanotic tumors that are rescued by mutation of *su(Hw)* in the *His2Av^{810}* mutant background. Double mutants of *su(Hw)$^{-}$* and *His2Av^{810}* also form supernumerary lymph gland lobes that are larger than in wild type. Our findings support a role for the Su(Hw) in the DNA damage response through the regulation of H2Av phosphorylation and suggest that Su(Hw) and H2Av also work together to ensure proper development of the lymph gland.

1280T **Altering phosphorylation of HP1a induces sterility and excites early cell death in *Drosophila melanogaster*** James C Walts[1], Nicole C Riddle[2] [1]Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

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Abstract

The Heterochromatin protein 1 (HP1) family are non-histone chromosomal proteins that are key factors in the formation of heterochromatin and in transcriptional regulation. HP1 proteins are found in many eukaryotic organisms including plants, animals, and fungi. HP1a from *D. melanogaster* was the first HP1 protein discovered, and it has been intensively studies for more than three decades. HP1a can act both as a repressor and an activator of transcription. Like many other proteins, HP1a undergoes post-translational modifications such as phosphorylation. However, little is known about the functions of HP1 post-translational modifications, including phosphorylation. To advance our understanding of HP1a's post-translational modifications, we produced two HP1a mutants that either mimic or block phosphorylation. Specifically, we replaced serines (S) 88/89/91 (S88/89/91) either with glutamic acid (E) to mimic permanent phosphorylation or with alanine (A) block phosphorylation. Using these mutant strains, we investigated how phosphorylation of HP1a impacts its known functions. Western blot analysis demonstrated that the phospho-mimic HP1a protein is stable but accumulates in decreased levels compared to wildtype HP1a. A position effect variegation (PEV) assay indicated that both phospho-mimic and phospho-block HP1a alleles act as suppressors of variegation. On the organismal level, we found that homozygous phospho-mimic HP1a mutants have a significant reduction in fertility for both males and females compared to heterozygous and wildtype animals. We observed a significant decrease in ovary size for the homozygous phospho-mimic HP1a mutant females when compared to heterozygous and wildtype animals. Parallel experiments with the HP1a phospho-block mutant strain are ongoing. Overall, our results show a significant fertility decrease, possibly due to the decreased ovary size in homozygous mutants, a decrease in overall mutant protein concentration with an increase in suppression of variegation when compared to a control. These results suggest that phosphorylation of HP1a proteins at the site we modified (S88/89/91) might have specific functions in the Drosophila germline.

1281T **The effects of *draper* gene knockdown in different tissues on ovarian function in *Drosophila melanogaster*** Pamela Yang, Cameron Dixon, Kimberly McCall, Jeremy Rojas Boston University

The gene *draper (drpr)* in *Drosophila melanogaster* is critical in cell clearance through its role as a phagocytic receptor. *drpr* plays a vital role in maintaining multiple tissues by clearing dead cells, bacteria, and debris. Recent research has shown decreased fecundity and increased egg retention when the *drpr* gene is absent. While mutants show defects in ovary maintenance, it is unclear which tissues are critical in this gene expression for whole body physiology. Using a tissue-specific RNA interference approach, dissection results showed a strong egg retention phenotype when *drpr* was knocked down with two different drivers: *elavGal4* (neurons) and *esgGal4* (gut). These initial results suggest that *Drosophila* with *drpr* deficient neurons or gut precursor cells have severe egg retention. Interestingly, another neuron driver tested, *nSybGal4*, did not have the same egg retention phenotype as *elavGal4* even though they targeted the same tissue. This may be due to the expression of *elav* primarily in early embryonic stages while *nSyb* is expressed mainly in adulthood, which questions the role of *drpr* expression in developmental stages for proper ovarian function. To further investigate this hypothesis, we are currently conducting temperature sensitive Gal80 experiments to introduce conditional knockdowns of *drpr* expression at different developmental stages in order to analyze their respective phenotypes. There are 3 transitional stages: larval, pupal, and adult, in which the *drpr* gene will be knocked down. We expect different severities in adult ovarian function with the larval transition group potentially having the highest egg retention phenotype. Further study will test this hypothesis and determine the severity of phenotype corresponding with each developmental stage *drpr* knockdown.

1282T **Drosophila Amus and Bin3 methylases functionally replace mammalian MePCE for capping and the stabilization of U6 and 7SK snRNAs** qiu peng, yiqing wang, ying xiao, Yikang Rong hengyang college of medicine, university of south china

U6 and 7SK snRNAs possess a 5' cap, believed to be essential for their stability and maintained by mammalian MePCE or Drosophila Bin3 enzymes. Although both proteins are required for 7SK stability, loss of neither destabilizes U6, casting doubts on the function of capping U6. Here we show that the Drosophila Amus protein, homologous to both proteins, is essential for U6 but not 7SK stability. The loss of U6 is rescued by the expression of an Amus-MePCE hybrid protein harboring the methyltransferase domain from MePCE, highlighting the conserved function of the two proteins as the U6 capping enzyme. Our new investigations in human cells establish a dependence of both U6 and 7SK stability on MePCE, resolving a long-standing uncertainty. While uncovering a division of labor of Bin3/MePCE/Amus proteins, we discovered a "Bin3-Box" domain present only in enzymes associated with 7SK regulation. Targeted mutagenesis confirms its importance for Bin3 function, revealing a possible conserved element in 7SK but not U6 biology.

1283T **Predicting evolutionary targets and parameters of gene deletion from expression data** Andre Luiz Campelo dos Santos, Michael DeGiorgio, Raquel Assis Florida Atlantic University

Gene deletion is traditionally thought of as a nonadaptive process that removes functional redundancy from genomes, such that it generally receives less attention than duplication in evolutionary turnover studies. Yet, mounting evidence suggests that deletion may promote adaptation via the "less-is-more" evolutionary hypothesis, as it often targets genes harboring unique sequences, expression profiles, and molecular functions. Hence, predicting the relative prevalence of redundant and unique functions among genes targeted by deletion, as well as the parameters underlying their evolution, can shed light on the role of gene deletion in adaptation. Here we present CLOUDe, a suite of machine learning methods for predicting evolutionary targets of gene deletion events from expression data. Specifically, CLOUDe models expression evolution as an Ornstein-Uhlenbeck process, and employs multi-layer neural network, extreme gradient boosting, random forest, and support vector machine architectures to predict whether deleted genes are "redundant" or "unique", as well as several parameters underlying their evolution. We show that CLOUDe boasts high power and accuracy in differentiating between classes, and high accuracy and precision in estimating evolutionary parameters, with optimal performance achieved by its neural network architecture. Application of CLOUDe to empirical data from *Drosophila* suggests that deletion primarily targets genes with unique functions, with further analysis showing these functions to be enriched for protein deubiquitination. Thus, CLOUDe represents a key advance in learning about the role of gene deletion in functional evolution and adaptation.

1284T **The Drosophila histone variant H2Av associates with insulator proteins to mediate an immuno-metabolic response** Bright Amankwaa[1], Mariano Labrador[2] [1]Biochemistry, Cell, Mol. Biology, University of Tennessee, Knoxville, [2]Biochemistry, Cell, Molecular Biology, The University of Tennessee, Knoxville

Dysregulation of chromatin insulators is associated with changes in gene expression and the onset of disease conditions, including cancer. Gypsy insulators in Drosophila contain binding sites for the Suppressor of Hairy-wing [Su(Hw)] protein and *su(Hw)* mutants display elevated number of chromosome breaks in dividing neuroblasts and in the germline, indicating an important role of Su(Hw) in the DNA damage response and the maintenance of genome integrity. Our recent genetic and bioinformatic analysis suggests a strong connection between gypsy insulators and the phosphorylated histone variant H2Av (γH2Av), which is critical for DNA damage repair. H2Av is also documented to suppress the Drosophila immune deficiency (IMD) pathway as evinced by the formation of melanotic masses in third instar of *His2Av* null mutants. Melanization is a first line insect-specific innate immune response that culminates in the enrichment of melanin at injury or infection sites. This work aims to understand the immune response role of H2Av and its connection with Drosophila insulator proteins. We found that the melanotic mass phenotype in H2Av null mutants is fully rescued in *His2Av-su(Hw)* double mutants. Our analysis revealed that fat body lipid droplets surround the melanotic masses occasionally becoming internalized within the melanotic mass, suggesting that the fat body tissue itself is the target for melanization. We also noticed that lipid droplets in the *His2Av* null mutants were significantly larger, but were reduced in the background of the *su(Hw)* mutant. Interestingly mutating any of the core gypsy insulator proteins or overexpression of H2Av in the fat body led to smaller lipid droplet formation. Mechanistically, we discovered that the elevated lipid droplets are linked to H2Av phosphorylation as the amount of lipid droplets in Drosophila S2 cells increases in response to treatment with okadaic acid, a known enhancer of phosphorylation. RNA-seq analysis associated the fat body phenotype with the expression of a number of metabolic genes including the Activity-regulated cytoskeleton associated protein 1(Arc1), which is an important regulator of lipid homeostasis and proper body fat storage. We propose a mechanism whereby H2Av cooperates with the canonical Drosophila insulator proteins to modulate an immuno-metabolic response via a pathway that has not yet been characterized.

1285T **Investigating mechanisms of gene expression as a function of dosage using multiple Drosophila species** Sierra Falcone[1], Leila Rieder[2] [1]Emory University, [2]Biology, Emory University

Histone proteins are essential for organization of the genome. Misregulation of these genes can cause aberrations in cellular divisions, leading to embryonic lethality. Canonical histone proteins in most metazoans are encoded by multigene families, but it is unclear how these genes are co-regulated to carefully control histone levels. Other multigene families, such as the rRNA genes, can provide clues: rRNA genes are controlled by epigenetic "nucleolar dominance" leading to expression of a subset of genes. Understanding how similar genes are differentially regulated is critical to our understanding of the relationship between transcriptional regulation and gene dosage.

I am investigating how expression of multiple histone genes is controlled using the powerful genetic model Drosophila. I am leveraging existing tools available in *Drosophila melanogaster*, as well as the novel histone locus organization found in other Drosophila species to investigate mechanisms of histone gene expression. I will utilize a parallel approach to profile dynamic developmental histone gene expression from a single locus using RNA sequencing. I will be utilizing the natural system of *Drosophila simulans*, in which there exists natural sequence variation in histone coding regions, and a transgenic system in *Drosophila melanogaster*. I will also profile locus-specific expression of histone genes in tissues with varying histone requirements. I will be using SNV FISH to determine relative abundance of histone transcripts using *Drosophila virilis*, which carries two asymmetrical histone loci, and *D. melanogaster-D. simulans* hybrids. These experiments will elucidate critical

mechanisms in gene family regulation by leveraging transgenic tools in *D. melanogaster* and underutilized genomes of non-melanogaster species.

1286T **Insights into sequential enhancer action through study of chromatin conformation dynamics at a single locus** Minh Tam Le[1], Leslie Dunipace[1], Dave Rumph[2], Angelike Stathopoulos[1] [1]Biology and Biological Engineering, California Institute of Technology, [2]Schmidt Academy for Software Engineering, California Institute of Technology

In the *Drosophila* embryo, gene expression patterns are often controlled by multiple enhancers acting in a coordinated manner to modulate expression over time. This study proposes that chromatin plays an important role in managing the sequential action of enhancers. Previous studies have shown that the early embryonic expression of the gene *brinker (brk)* is controlled by two cis-regulatory elements that act sequentially during cellularization. At this stage, the promoter proximal element (PPE) regulates the switch between the early 5' enhancer towards the late 3' enhancer resulting in a change in *brk* gene expression from narrow to broad (Dunipace et.al, *Developmental Cell*, 2013). We hypothesized that the PPE regulates sequential enhancer action by controlling chromatin conformation dynamics. This study proposes a new method to measure local chromatin changes over time utilizing Oligo-paint DNA fluorescence in situ hybridization (FISH) and an in-house computational pipeline to analyze co-localization of three DNA segments that vary on the linear DNA strand by ~10 kB. This imaging and computational approach is able to extract 3D conformations within co-localizing triplet spots. This set of tools will facilitate our study of how sequential-action of enhancers is regulated by a query of chromatin conformations in space and time within embryos. Our preliminary results have identified a subtle change in chromatin structure over developmental time at the *brk* locus. Specifically, in wild-type embryos, our results point to an increase in chromatin compaction in fully cellularized embryos as compared to earlier stages suggesting the 3' enhancer more frequently interacts with the PPE at this later developmental stage. Future experiments include assay of other mutants that have been shown to regulate *brk* gene expression to test whether they affect chromatin conformation. To start, we will utilize our developed tool to test the hypothesis that the PPE affects chromatin conformation by using a mutant that has a 800 base pair deletion within the PPE region. By assaying this and other mutant embryos, this approach also may help to identify the protein effectors that control chromatin dynamics and impact sequential enhancer action.

1287T **Identification of a candidate *akirin* enhancer sequence** Miranda Forman-Grimm, Georgette-Vanelle Wandji, Scott J. Nowak Department of Molecular and Cellular Biology, Kennesaw State University

Akirin, a small nuclear protein with conserved function across eukaryotes, is a critical determinant in the development of functional and robust cardiac and skeletal patterning and musculature. Akirin serves as a transcription cofactor by acting as a bridge or link between transcription factors such as Twist with chromatin remodeling complexes to ensure that Twist functions appropriately during transcription. If Akirin function is impaired, the resulting muscle patterning and structure is greatly impacted. We have identified a short sequence within the first intron of *akirin* that is highly conserved among closely related *Drosophilid* species. We are evaluating this sequence for possible promoter or enhancer activity. This evaluation is being performed using a variety of *in vivo* and *in vitro* techniques, both in live *Drosophila* embryos, as well as in cultured S2 cells. We have determined that a likely candidate enhancer sequence does indeed occur within this conserved element and are investigating a number of candidates that regulate this particular DNA sequence for *akirin* expression.

1288T **Investigating mechanisms of *D. melanogaster* histone locus body initiation and maintenance.** Nicole Roos[1], Greg Kimmerer[2], Leila Rieder[2] [1]Biology, Emory University, [2]Emory University

Histone proteins are essential for compaction and regulation of the eukaryotic genome. Dysregulation of histone gene expression leads to aberrant development and lethality. In animals, genes encoding the histone proteins are commonly clustered at loci. The histone locus body (HLB) is a collection of factors that localize to histone loci and regulate histone gene expression. By studying initiation and maintenance of the HLB, we gain insight into how unique genes are targeted by specialized transcription factors for specialized regulation. We are employing the powerful model system *Drosophila melanogaster* in which we leverage histone transgenes that recruit HLB factors, allowing us to manipulate *cis* regulatory elements and determine the effects on HLB formation and histone expression. In flies, the HLB is initiated during early embryogenesis. The HLB is then maintained at the single locus in mature tissues and throughout cell divisions. To test whether the cis elements that are critical for HLB initiation are also important for maintenance, we employ a transgenic system where we will detect HLB maintenance using a combined DNA FISH and immunofluorescence protocol. To test whether HLB initiation can occur in non-embryonic cells, we will combine transgenes and mitotic recombination in wing discs. Overall, studying HLB initiation and maintenance allows us to illustrate how and when histone proteins are regulated and in what contexts factors target these crucial genes for regulation.

1289T **Transcription factor localization to the *D. melanogaster* histone genes** Connor Smith[1], Thomas O'Haren[2], Leila

Rieder[3] [1]Emory, [2]Emory Genetics and Molecular Biology, [3]Emorty

Eukaryotic cells need to fit a large amount of DNA into a very small space. Histones are important proteins that condense DNA in the nucleus, influencing gene expression. The histone genes themselves are rigorously controlled; even small changes in histone biogenesis may lead to global defects in pre-mRNA splicing, temporal errors in cell division, whole genome duplications, genomic instability, and embryonic lethality. In metazoan genomes, the histone genes are typically repetitive and clustered and attract a myriad of transcription factors. These TFs, along with histone mRNA processing factors, collectively form the histone locus body (HLB). HLB TFs are largely discovered by chance, and the inventory of known factors is not comprehensive. This leaves current knowledge of the HLB limited – both compositionally and systematically. A complete list of all HLB factors is required for better understanding of the mechanisms of histone gene regulation.

Through expansion of a bioinformatic screening mining publicly available ChIP-seq data, we discovered several exciting candidates that show localization to the histone gene array. To confirm candidate localization *in vivo*, we will perform polytene chromosome immunostaining. Additionally, we will drive depletion of candidate through RNA interference using available transgenic fly lines to determine if candidate removal results in a change of histone transcript levels.

To extend our screening efforts, we will also be performing a Yeast-2-Hybrid on a protein that solely targets the histone array. Multi-sex comb (Mxc) is an HLB scaffolding protein that is conserved from humans to Drosophila and only targets the histone locus. Due to this specific localization, we hypothesize that examination of Mxc interactors will identify novel HLB TFs. This method uniquely provides us with a nonrandom, unbiased method of discovering transcription factors that localize to the histone locus These experiments in tandem will expand our understanding of how factors that compose the HLB interact and orchestrate histone production.

1290T **Functional clusters of the *even-skipped* stripe 2 enhancer in Drosophilidae and Sepsidae** Alan U Sabino[1], Ah-Ram Kim[2,3,4,5], Pengyao Jiang[2,6], Chan-Koo Kang[5], Michael Z Ludwig[2], David H Sharp[7], Alexandre F Ramos[8], John Reinitz[2,9,10] [1]Departamento de Radiologia e Oncologia, Universidade de Sao Paulo, [2]Department of Ecology and Evolution, University of Chicago, [3]Department of Biochemistry and Cell Biology, Stony Brook University, [4]School of Life Science, Handong Global University, [5]School of Creative Convergence Education, Handong Global University, [6]Department of Evolutionary Biology, Arizona State University, [7]Los Alamos National Laboratory, [8]Escola de Artes, Ciencias e Humanidades, Universidade de Sao Paulo, [9]Department of Statistics, University of Chicago, [10]Department of Molecular Genetics and Cell Biology, University of Chicago

Metazoan genes contain large segments of regulatory DNA which is divided into functional subunits known as enhancers. Exactly how the DNA sequence in open regions of chromatin encodes a pattern of expression remains an unsolved problem, particularly in the case of ”billboard” enhancers, in which the spacing and number of binding sites may vary. In this study, we report progress in elucidating these rules by the analysis of a collection of enhancers from a variety of dipteran species. We sought to understand how reporters driven by enhancers from the Drosophilid species *D. yakuba* and *D. pseudoobscura* and the Sepsid species *C. cynipsea* and *T. putris* drive *eve* stripe 2 expression at the native position when placed in *D. melanogaster* embryos, despite significant sequence changes in the two Drosophilid species and even larger ones in the two Sepsid species. Using an *in silico* model informed by genetic experiments, we show how these phenomena can be understood in terms of the molecular interactions that control stripe 2 expression. We find that stripe 2 is driven by Hb bound at two sites, each under the influence of clusters of neighboring bound coactivators and repressors. We further show how the role of Bcd as a coactivator in conjunction with a “shadow” Hb binding site gave rise to the classic picture that Bcd, rather than Hb, is the main activator of stripe 2. The robustness of this result and other results shown is supported by a new computational algorithm for analyzing large ensembles of models. In the Drosophilids, some of these interactions among bound transcription factors are conserved across species, while others are lost and replaced by new compensatory interactions which result in the same output. Certain important features of the clusters appear in both Drosophilidae and Sepsidae, but Bcd is partially replaced by Cad as a coactivator in the Sepsidae. We present experimental results confirming this finding. These clusters are an essential feature of the *cis*-regulatory code for transcriptional control. We further report on the extension of this method of analysis to a wider class of enhancers and dipteran species. The functioning of these clusters involves the whole group of binding sites and is insensitive to small changes in the spacing, number, or affinity of individual sites.

1291T **Regulation of Oogenesis by JNK Signaling** Sakhee Thakkar, Evelyn Tello, Jennifer Mierisch Loyola University Chicago

Oogenesis is the process by which female gametes are formed, and begins in the germarium where germline and somatic stem cells divide to support the formation of new egg chambers. Studying regulators of oogenesis is important for understanding how genes that are mutated can lead to infertility. We are interested in exploring further the role of the Jun N-terminal kinase (JNK) Signaling pathway and related proteins in oogenesis. A combination of RNA interference (RNAi) and overexpression was

used to manipulate expression of the JNK family proteins in somatic follicle cells to examine the effect of altered JNK signaling on eggs. While overexpression of activated Hemipterous, a JNKK, severely disrupted egg production, and RNAi of basket, which encodes JNK, and jun-related antigen (jra), and raw resulted in milder defects in egg shape. We are testing if these misshapen eggs are viable and able to produce offspring via hatching assays.

We are further exploring the function of these genes in oogenesis by manipulating gene expression followed by immunohistochemistry to understand how altered JNK signaling leads to defects in oogenesis in Drosophila. We expect these findings can be applied more broadly to how JNK signaling regulates oogenesis across Drosophila species.

1292T **Functional Characterization of ZAD Gene Family in Drosophila melanogaster.** Riley T Reed[1], Jack D Jurmu[2], Andrew M Arsham[1] [1]Biology, Bemidji State University, [2]University of Utah

Many vital eukaryotic developmental processes hinge on the formation and regulation of heterochromatin. Broadly, heterochromatin is gene poor and a tightly compressed structure of chromatin which restricts the transcription of local genes. While much genomic heterochromatin (for example centromeres and telomeres) is constitutive, it can also be induced as a genomic defense against invasive DNA, and facultative heterochromatin regulates developmental gene regulation. Recently, genes in the ZAD family have been identified as key regulators of heterochromatin in Drosophila melanogaster, however many are uncharacterized. Here we identify heterochromatin regulatory functions for currently uncharacterized ZAD genes by measuring the effect of eye-specific RNAi knockdown on Position Effect Variegation (PEV), a proxy for heterochromatin-mediated reporter gene silencing. We measured the effect of ZAD gene knockdown on reporter gene expression and compared the impact of knockdown on three distinct heterochromatin types: the classic wm4 mutant in which PEV is mediated by spreading of pericentric heterochromatin on the X chromosome; a line in which a white reporter is inserted in the largely heterochromatic 4th (or dot) chromosome; and a line in which heterochromatin silencing has been triggered by the presence of a 10 kb tandem array of repeats from the e. coli lac operon. Our data define heterochromatin regulatory function for uncharacterized ZAD genes and suggest that these functions may be further categorized by the specific type of heterochromatin with which they interact.

1293T **Repetitive DNA triggers *cis* and *trans* gene inactivation in *Drosophila*** Han Rhee, Andrew M Arsham Biology, Bemidji State University

Heterochromatin is a key genomic defense against transposons. In *Drosophila,* insertion of repetitive DNA can trigger the conversion of euchromatin to heterochromatin, stochastically silencing nearby reporters by recruiting HP1 and resulting in variegated gene expression. While this phenomenon is typically studied in *cis,* examples of *trans*-inactivation, in which a silenced allele can epigenetically inactivate an expressed allele on a paired homologous chromosome, have also been described. In the context of *Drosophila* eye color reporter genes, this produces the counterintuitive effect of a normally recessive biosynthetic phenotype (loss of pigmentation leading to white eyes) becoming epigenetically dominant. The mechanisms and regulatory implications of *trans*-inactivation remain poorly understood. Here, we document a new experimental system for studying *trans*-inactivation at multiple euchromatic chromosomal locations using transgenic reporter alleles that have been silenced in *cis* by a 256-copy array of the *e. coli* lac operator (lacO) sequence. Surprisingly, we found that the epigenetically silenced allele can transmit that silenced state to a non-silenced allele at the same location on the homologous chromosome. Pigmentation levels in *trans*-inactivated flies is intermediate between the silenced and non-silenced alleles suggesting a dynamic balance between the biochemically dominant pigment biosynthesis gene and the epigenetically dominant silenced state. Identical silenced insertions at other genomic locations are being tested to investigate distance-dependence and the molecular mechanisms of this poorly understood phenomenon.

1294T **Topoisomerase 3B-TDRD3 Complex Regulates Metabolism and Development in *Drosophila melanogaster*** Anantha Korrapati, Tianyi Zhang, Weidong Wang National Institute on Aging

Topoisomerases are required during transcription to release DNA topological stresses, and Topoisomerase 3β (Top3B) is the only dual-activity enzyme that can change the topology of both DNA and RNA. Top3B forms a stable complex with Tudor domain containing protein 3 (TDRD3). Top3b/TDRD3 complexes are mainly localized in the cytoplasm and integrated into stress granules under oxidative stress conditions. Mutations in Top3B have been associated with cognitive impairments and developmental problems in humans. However, Top3b-KO flies and mice survive to the adult stage without obvious developmental defects. To understand Top3B-TDRD3's physiological role in development and metabolism, we performed gain-of-function analysis with *Drosophila melanogaster* larvae. We observed that larvae with moderate overexpression (OE) of Top3b and TDRD3 survive to the adult stage while those with high-level OE die at the pupal stage. To investigate the impacted gene pathways, we utilized RNA-seq to analyze the transcriptome of the flies with different levels of OE. Compared with the negative control, the OE flies have up-regulation of genes involved in glycolic-related metabolism and transport as well as down-regulation of genes involved in developmental, nervous system, and immune functions. We will further study the

OE-affecting tissues by analyzing tissue enrichment of the up- and down-regulated genes via RNA-seq. With these results, we will compare the transcriptomes of WT, Top3B-KO, and TDRD3-KO larvae to identify Top3b/TDRD3 targets. Meanwhile, we are doing RNA-seq with Top3b-KI (dead enzyme) and Top3b-KO mouse kidneys under normal and starved conditions to evaluate the effect of Top3B on metabolism. Our preliminary results show that several pathways and genes related to metabolism display altered expression in kidneys of Top3b mutant animals under starvation conditions. Overall, this project will elucidate the role of the Top3B-TDRD3 complex in metabolism and development and its relevance to aging.

1295T **Investigating the role of Hippo signaling in *Drosophila melanogaster* cuticle pigmentation and dopamine metabolism** Shelley B Gibson[1,2], Samantha L Deal[2,3], Shinya Yamamoto[3,4] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Program in Developmental Biology, Baylor College of Medicine, [4]Neuroscience, Baylor College of Medicine

Many biological processes are highly conserved between humans and *Drosophila melanogaster*, two of which are the dopamine (DA) synthesis pathway and the Hippo signaling pathway. Both pathways are implicated in and cause a wide range of diseases or disorders. Understanding the mechanisms by which DA synthesis is regulated is necessary to develop novel treatments for those affected by dysregulation of DA signaling pathways, including Parkinson's disease, schizophrenia, and mood disorders. Although the downstream signaling cascades of DA signaling in postsynaptic neurons has been characterized well, relatively few genes and proteins are known to regulate DA levels in DArgic neurons. The two enzymes that mediate DA synthesis, Tyrosine hydroxylase (TH) and Dopa decarboxylase (Ddc), are conserved between *Drosophila* and humans. In addition to its role in the nervous system, DA is used as a precursor to melanin in *Drosophila* cuticle pigmentation. We identified four kinases of the core Hippo signaling pathway, *hippo*, *salvador*, *mats* and *warts*, as well as the effector *yorkie* in the same process, thus implicating all five key genes of the canonical Hippo signaling pathway as novel regulators of pigmentation and DA levels. This pigmentation phenotype observed with *warts* knockdown (KD) was rescued with the human homolog, *LATS1*, meaning that the cuticle could be used as a screening tool to identify human disease causative variants in many different Hippo signaling genes. Additionally, KD of *warts* in DArgic cells leads to increases of DA in the fly head but not the brain, suggesting our methods are capturing cuticle only changes of DA levels due to inhibition of Hippo signaling. Because conditional KD of *warts* and overexpression of *yorkie* in the adult stage led to no change in DA levels, inhibition of Hippo signaling may alter dopamine levels during development, when TH and Ddc have highest levels of expression before cuticle melanization is established. Based on the data obtained so far, I hypothesize that the Hippo signaling pathway regulates melanin levels through key enzymes involved in DA synthesis, such as TH and Ddc and the fly cuticle may also be used as a screening tool for disease causing Hippo signaling variants. These studies will expand our knowledge of Hippo signaling targets in *Drosophila* cuticle pigmentation though dopamine regulation, and whether this link is conserved in mammalian DArgic cells, including human.

1296T **A model system to study Kabuki syndrome utilizing KMT2D histone H3 lysine 4 methylase proteomics during cranial neural crest osteoblast differentiation** Sara Vardabasso, Karl B. Shpargel Genetics, University of North Carolina at Chapel Hill

Kabuki syndrome is a human disorder that presents with characteristic craniofacial phenotypes including facial hypoplasia, a depressed nasal tip, ocular abnormalities, and cleft palate. Kabuki syndrome results from mutations in KMT2D and KDM6A enzymes that regulate histone methylation. KMT2D is a histone H3 lysine 4 methylase involved in enhancer activation. We have previously modeled facial dysmorphism of Kabuki syndrome in the mouse utilizing neural crest specific deletion of KMT2D. Neural crest cell (NCC) specific deletion of KMT2D results in deficiencies in osteoblast differentiation that lead to alterations in the formation of anterior facial bones. We have now developed KMT2D mutations in neural crest cell culture to identify functional mechanisms of histone methylation regulates osteoblast differentiation. KMT2D mutant neural crest cells exhibit strong reductions in H3K4 mono and di-methylation. When placed in osteogenic media, the KMT2D mutant stem cells are deficient in differentiating to osteoblast lineages. To further understand KMT2D mechanisms, we propose to use a knock in system for proximity biotinylation to identify associated KMT2D co-factors during osteoblast differentiation. We have inserted the APEX peroxidase on to the endogenous KMT2D carboxy-terminus. Through oxidation of phenol derivatives, APEX will catalyze the formation of reactive unstable radicals, which have a high affinity for electron-rich amino acids. This will result in proximal protein labeling with biotinylation. Proximity-dependent biotinylation followed by streptavidin capture and mass spectrometry allows for isolation and identification of protein complexes interacting with KMT2D in differentiating neural crest cells. We conclude that KMT2D function is required for H3K4 methylation, NCC osteoblast differentiation, and facial bone formation. These collective proteomics experiments will lead to a better understanding KMT2D cooperation with chromatin and transcription factor complexes during osteoblast differentiation.

1297T **Determining the cause of hemimethylation at secondary DMRs associated with imprinted genes** Tamara L. Davis[1], Jordan Ellis-Pugh[2], Jacqueline Saulnier[2], Jaclyn Lo[2], Courtney Link[2], Sophia Gibson[2], Isabel Oalican[1], Julia Kesack[1], Naideline Raymond[2] [1]Biology, Bryn Mawr College, [2]Bryn Mawr College

The monoallelic expression of imprinted genes is regulated by parent of origin-specific differential DNA methylation. Imprinting control regions, or primary DMRs, regulate epigenetic profiles and expression patterns across imprinting clusters. In contrast, differential methylation at secondary DMRs influences the expression of individual imprinted genes. Unlike primary DMRs, secondary DMRs display considerable variability in their DNA methylation patterns and have high levels of hemimethylation. We hypothesize that the high level of hemimethylation is the result of 5-hydroxymethylcytosine enrichment at these loci. We tested this hypothesis by subjecting mouse genomic DNA to parallel bisulfite and oxidative bisulfite mutagenesis reactions. Loci associated with imprinted and non-imprinted genes were amplified from the mutagenized DNA and subjected to NextGeneration amplicon sequencing. This approach allowed us to determine the amount of 5hmC at each locus through a subtractive method as the bisulfite reaction identifies 5-methylcytosine and 5-hydroxymethylcytosine while the oxidative bisulfite reaction only identifies 5-methylcytosine. Our preliminary results illustrate low levels of 5-hmC at primary DMRs and non-imprinted loci. Secondary DMRs show less consistent results, but generally indicate that there may be 5-hmC enrichment at paternally methylated secondary DMRs. Additional studies are underway to better understand the distribution of 5-hmC at differentially methylated sequences associated with imprinted genes.

1298T **Molecular basis of seasonal adaptation in mammals** liang Ren[1,2], Junfeng Chen[1], Kousuke Okimura[2,3], Yang Chen[3], Fukawa Kai[1,2], Sumiyama Kenta[2,4], Takashi Yoshimura[1,2] [1]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, [2]Graduate School of Bioagricultural Sciences, Nagoya University, Japan, [3]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Japan, [4]Laboratory of Mouse Genetic Engineering, RIKEN Center for Biosystems Dynamics Research (BDR), Japan

Rhythms of life on earth are shaped by the seasons. Accordingly, various physiological functions such as hormonal secretion, metabolism, growth, immune function, and reproduction show profound seasonal changes in animals including humans. Morbidity in humans due to cardiovascular disease, influenza, and psychiatric diseases is also seasonally regulated and peaks in winter. However, their underlying molecular bases remain unknown. Our transcriptome analysis in rhesus macaques (*Macaca mulatta*) suggested the involvement of GA binding protein (GABP) in the regulation of seasonally oscillating genes. In this study, we examined the siRNA knockdown in macaque fibroblasts and revealed the involvement of GABP in the regulation of seasonally oscillating genes. To test the *in vivo* role of GABP in the seasonal adaptation, we aimed to generate GABP mutant mice by CRISPR/Cas9 method. Most of the commonly used laboratory mouse strains genetically lack melatonin that is believed to be important for seasonality. However, both melatonin-proficient CBA/N mice and melatonin-deficient C57BL/6N mice showed clear seasonality. For instance, exposure to summer- and winter-mimicking condition induced significant change in organ weight in multiple tissues under winter-mimicking condition. Histological analyses demonstrated differences in cell division and cell size between summer and winter conditions. Based on these results, we next examined organ weight in *Gabpb1* mutant mice exposed to summer- and winter-mimicking condition. As a result, *Gabpb1* mutant mice showed impaired seasonal responses, reflecting in the reduction in seasonal differences in tissue weight, cell division and cell size. These results suggest possible involvement of GABP in the seasonal adaptation in mammals.

1299T **Elucidating the mysterious function of noncanonical DNA methylation in the brain** Mandy Eckhardt, Tiffany Chin, Eric Sun UT Southwestern Medical Center

DNA methylation is an evolutionarily ancient chemical modification of DNA bases essential for development, and it typically occurs at cytosine-guanine dinucleotide sequences (mCG) in mammals. More recently, other forms of DNA methylation, most notably at cytosine-adenine sites (mCA), have been observed in specific cell types. Interestingly, mCA is most abundant in neurons where mCA levels exceed that of mCG, and this methylation pattern has not been observed in any other cell type. Further, DNA methylation is inherently mutagenic, yet it is evolutionarily conserved in most organisms, thus highlighting the fundamental and critical role of DNA methylation in biological systems. Despite its abundance in the brain, only a few proteins have been identified in the mCA pathway. DNA methyltransferase 3A (DNMT3A) facilitates all mCA deposition in the brain, but only one mCA-binding protein is known: methyl-CpG binding protein 2 (MECP2). Very little else is known about the mCA pathway, and mCA function remains unknown. However, the presence of neurodevelopmental disorders caused by mutations in *DNMT3A* or *MECP2*, including autism spectrum disorder, overgrowth syndromes, Rett syndrome, and *MECP2* duplication syndrome, underscore the importance of investigating mCA. One way to elucidate the mCA pathway is to determine proteins that preferentially interact with mCA. To do so, we utilized a proteomic screen to identify mCA-binding proteins. Consistent with current knowledge, MECP2 emerged as the most abundant protein found in the mCA pull-downs. With this screen, we identified a novel putative mCA-binding protein. This protein is not well-studied, as its function, structure, and role in the brain are unknown. Publicly available data indicates expression of this putative mCA-binding protein correlates with mCA occupancy, as it is only expressed in the brain, and is expressed primarily in neurons. Further, like mCA, this protein is conserved throughout vertebrates, suggesting both are important for vertebrate brain function and/or development. Current efforts seek to characterize the relationship between mCA and this candidate mCA-interacting protein by determining its DNA binding pattern in vivo in the context of mCA using Cleavage Under Targets and Release Using Nuclease (CUT&RUN), identifying

interacting proteins via co-immunoprecipitation, and characterizing molecular and behavioral phenotypes in a knockout mouse line.

1300T **Exploring the systems genetics of Age-related Macular Degeneration in Diversity Outbred mice** Abdulfatai Tijjani[1], Selcan Aydin[2], Gayle Collin[2], Juergen Naggert[2], Steven Munger[1] [1]Lab - Steven Munger, The Jackson Laboratory, [2]The Jackson Laboratory

Age-related Macular Degeneration (AMD) is one of the leading causes of irreversible blindness among the aging population and requires urgent therapeutic intervention. Genome-wide association studies in humans have implicated numerous genetic variants, however it's unclear how these primarily non-coding variants influence disease susceptibility. Mapping expression quantitative trait loci (eQTLs) in relevant disease tissues in genetically diverse mice enables us to link genetic variants to their proximal effects on gene expression and distal effects on disease outcomes, and in the process can help identify novel gene candidates, biomarkers associated with disease susceptibility, and potential drug targets. Here, we characterized and compared the retinal pigment epithelium (RPE) and retina transcriptomes of 190 12-week-old Diversity Outbred (DO) mice raised on a standard chow or Western diet. We quantified the expression of 15,000 transcripts and mapped eQTLs in each tissue. Interestingly, we identified two significant eQTL hotspots on Chromosomes 2 and 12 specifically affecting the RPE of DO mice fed the Western diet. Many hotspot target genes are involved in ocular development and neurological processes, potentially suggesting a genetic link between AMD and neurodegeneration. Despite these expression differences, it should be noted that these young mice displayed no overt AMD physiology, suggesting that AMD-associated effects on the RPE transcriptome can be observed long before onset of the disease. Future studies will seek to identify the causal genes underlying the eQTL hotspots and to extend our analysis to predict combinations of DO founder strain alleles that better model the full spectrum of human AMD phenotypes

1301T **Specific genes regulated by histone deacetylase Hst1 contribute to *Candida glabrata* survival under stress** Bowen Liu[1], Laura Rusche[2] [1]University at Buffalo, SUNY, [2]State University of New York at Buffalo

The yeast *Candida glabrata* is the second most common cause of candidiasis in North America and Europe. During infection, this opportunistic fungal pathogen encounters stresses such as low NAD+ availability and anti-fungal azoles. Genes that allow *C. glabrata* to respond to these stresses are repressed by the SUM1 complex, including the gene encoding NAD+ precursor transporters, *TNA1*, and the gene controlling drug resistance, *PDR1*. SUM1 complex contains a histone deacetylase Hst1 and a transcription factor Sum1. Many targets of SUM1 complex are related to stress response. However, it remains unknown the full list of genes regulated by SUM1 complex and whether they affect each other.

To find out genes physically associated with SUM1 complex, we are conducting ChIP-exo with *HST1-V5* and *SUM1-V5* strains. We completed pilot ChIP-qPCR assays on *TNA1* and *PDR1* promoters. Eventually, we will utilize RNA-seq to identify genes changing expression levels in the absence of *HST1* or *SUM1*. To do so, we made *HST1*-N293A and *sum1*Δ strains and conducted pilot RT-qPCR assays to check expression level of *TNA1* and *PDR1*.

1302T **Genes affected by NaCl during root development in *Arabidopsis thaliana*** Leonard Pysh, Davin Stevenson Biology, Roanoke College

Sodium chloride (NaCl) in soil has negative impacts on plant growth, reducing crop yields. Although soil salination occurs naturally, it has increased substantively due to modern agricultural practices. An improved understanding of the basis for the negative impacts of NaCl on plant growth is important to developing rational strategies to address and ameliorate these effects. While many investigators have studied the effects of NaCl on plant growth, relatively few have focused on roots, and most of the studies of NaCl responses in roots have been performed in the model plant *Arabidopsis thaliana* using seedlings that have been germinated and grown in the light. Light is known to affect root development, and initial analyses indicate that root responses to NaCl are affected by light. To understand better the specific effects of NaCl on root development, we have begun to identify a set of genes that are either up- or down-regulated in roots when grown on media containing elevated levels of NaCl. The longer-term goal is to use the regulatory regions of these genes to develop transcriptional reporter lines that will provide insights into the specific cellular targets of NaCl in plant roots.

1303T **The effects of mutations on gene expression and alternative splicing** Marelize Snyman[1,2], Sen Xu[2,3] [1]Dermatology, UT Southwestern Medical Center, [2]Biology, University of Texas at Arlington, [3]University of Missouri

Exploring the intricate connection between mutations and their genomic and phenotypic consequences has long been a fundamental pursuit within evolutionary biology. However, only a few studies have investigated the effects of mutations on gene expression and alternative splicing on the genome-wide scale. In this study we therefore aim to bridge this critical knowledge gap by leveraging comprehensive data from whole-genome sequencing and RNA sequencing across 16 obligately

parthenogenetic Daphnia mutant lines to investigate the impact of ethyl methanesulfonate-induced mutations on gene expression and alternative splicing. Through rigorous analysis of mutations, changes in expression, and alternative splicing patterns, our findings reveal that trans-effects overwhelmingly account for the variation in gene expression and alternative splicing between wild-type and mutant lines. In contrast, cis mutations exert a limited influence on gene expression and don't consistently lead to alterations in gene expression. Additionally, we highlight a significant correlation between differentially expressed genes and mutations occurring within exons, underscoring the pivotal role of exonic mutations in driving changes in gene expression.

1304T **Directionality of transcriptional regulatory elements reveals architectural, evolutionary and functional dynamics** You Chen, Sagar R Shah, Alden Leung, John T. Lis, Andrew Clark, Haiyuan Yu Cornell University

Divergent transcription has been demonstrated as a critical mark of active transcriptional regulatory elements (TREs). However, in addition to divergent elements (each produced divergent transcripts seen as a pair of peaks on opposite strands and within 300 bp), a considerable number of unidirectional elements (only producing transcripts from one strand) have been detected from PRO-cap data and might also function as TREs. These unidirectional elements are largely overlooked and their identity and function remain elusive.

A total of ~40,000 TREs were identified from K562 PRO-cap data and divided into proximal (within 500 bp, putative promoters) and distal (outside 500 bp, putative enhancers) categories based on their distances to transcription start sites (TSSs) of known genes. Systematic comparison of epigenomic features and transcription factor binding profiles revealed distinct architecture models for divergent and unidirectional elements. Detailed inspection of sequence content confirmed that unidirectional TREs are a true biological entity, not simply a result of experimental artifacts, low sequencing depth or poor mappability. Furthermore, we found that both divergent and unidirectional TREs are significantly older and subject to stronger selective constraints than the random genomic background, indicating that both classes of TREs may have been maintained due to their functions. In the distal category, unidirectional elements tend to be younger, with fewer evolutionary constraints compared with their divergent counterparts, which suggests that they arose more recently in evolution and represent a distinct group of functional units. Consistent with this, we found that unidirectional elements were less likely to have target genes predicted by the ABC model and had higher cell-type specificity. PRO-cap data of CD4+ T cells from human, rhesus macaque and baboon also revealed dynamic changes in directionality across species.

Altogether, the elucidation of TRE transcriptional directionality will help build a finer architecture model and enable a better understanding of evolutionary and functional dynamics of TREs.

1305T **Investigating the genomic, transcriptomic, and metabolic changes in response to DNA replication stress in the telomeres of *Saccharomyces cerevisiae*** Taizina Momtareen West Virginia University

Telomere shortening is associated with aging of healthy cells and telomere elongation is a hallmark of cancer cells. About 10-15% of cancer cells use a type of DNA recombination called Alternative lengthening of telomeres (ALT) to extend their telomeres instead of using telomerase. Similarly, telomerase-mutants of yeast (*S. cerevisiae)* proliferate using DNA recombination, whereby they undergo aging to reach replicative senescence, but few can resume cell division after that, becoming post-senescence survivors. It is unclear what occurs at a molecular level that enables these cells to revive themselves from senescence and resume proliferation. In general, as cells age, they lose mitochondrial function and accumulate damaged lipids, proteins, and DNA. One hypothesis of aging is that it is triggered by this accumulated damage. Since the telomerase-mutants are essentially reversing aging to become survivors, it can be hypothesized that their transcriptional and metabolic profiles go through major changes to enable the cells to divide again, despite the wear and tear of aging. For example, research in survivors has shown increased transcription of energy production genes, indicating a higher demand for energy to sustain the cells. However, how this variation in gene expression causes the mitochondria to generate more energy and help the telomerase-mutants to become survivors is unknown. The goal of this research is to elucidate such variations occurring in telomerase-mutants that could potentially cause them to turn into survivor cells. My research seeks to determine this by 1) evaluating and interpreting the changes in physiology and energy production processes of cells lacking telomerase, and (2) determining the transcriptional response to telomerase deletion, senescence, and telomerase-independent telomere lengthening. The data generated from this work will result in a broader view of the telomerase mutants and enable us to draw parallels with aging and ALT cancer cells.

1306T **New insights into the regulation of gene expression by the SAGA/NuA4 co-activator complexes in *Candida albicans*** Gabriela MN Librais[1], Samuel Stack-Couture[1], Yuwei Jiang[1], Iqra Razzaq[2], Matthew D Berg[3], Julie Genereaux[1], Christopher J Brandl[4], Rebecca S Shapiro[2], Patrick Lajoie[1] [1]Anatomy and Cell Biology, The University of Western Ontario, [2]University of Guelph, [3]University of Washington, [4]Biochemistry, The University of Western Ontario

Fungal infections and the emergence of drug-resistant strains are a growing worldwide threat to human health. Therefore, identifying new targets to increase susceptibility of fungal pathogens to current available therapies is crucial in developing effective treatment against fungal infections. Tra1 is an essential component of both the SAGA-and NuA4 transcription acetyltransferase complexes that acetylate histones and non-histone proteins to regulate gene expression in response to various stresses, including antifungal drug treatment. As a PIKK family member, Tra1 is characterized by a C-terminal phosphoinositide 3-kinase (PI3K) domain. Like other PIKK family members such as TOR, Tra1 should therefore be druggable. We previously identified specific residues within the putative ATP-binding cleft of the *S. cerevisiae* Tra1 PI3K domain that regulate assembly and function of both SAGA and NuA4 (**Berg *et al*., 2018, G3**). The resulting mutant termed tra1Q3 displays increased susceptibility to multiple stressors, including multiple antifungal compounds such as caspofungin and fluconazole. An equivalent mutant generated using CRISPR-Cas9 genome editing in the pathological yeast *C. albicans* displays unsuspected phenotypes. While , tra1Q3 cells show reduces virulence and increased sensitivity to caspofungin (**Razzaq *et al.* , 2021, Genetics**), it displays decreased susceptibility to azoles, suggesting a rewiring of Tra1 functions across yeast species (**Librais *et al.* , 2023 G3**). Moreover, inositol auxotrophy, one the phenotypes typically associated with SAGA mutants in budding yeast, is not recapitulated in C. albicans, further highlighting functional differences between the two yeast species. Thus, we are investigating how the assembly, composition and functions of the SAGA/NuA4 complexes regulate global protein acetylation to control *C. albicans* virulence and drug resistance. Supported by CIHR.

1307T **Unveiling a Novel Role for a Transcription Factor in Yeast RNA Splicing** Siddhant Kalra, Joseph David Coolon Biology, Wesleyan University

RNA Splicing is a fundamental cellular process in eukaryotic organisms, characterized by a multitude of intricately regulated molecular processes governed by the spliceosome protein complex. This complex orchestrates the precise excision of intron sequences from a pre-messenger RNA (pre-mRNA) molecule. Alternative splicing of pre-mRNAs can enable the production of multiple RNA isoforms from a single gene allowing cells the capability to finely regulate not only which isoforms are transcribed and further translated into proteins but also the quantity of those proteins dramatically increasing the potential protein products of the genome.

In the budding yeast Saccharomyces cerevisiae, only a small number of genes (~3%) require splicing and typically only contain 1 intron. As a result, prior work on alternative splicing of these genes has been limited. While only a few genes have introns, those that do are highly transcribed and therefore give rise to 1/3rd of all pre-mRNA transcripts in the cell. By performing RNA sequencing analysis, we elucidate a novel role of an essential transcription factor (TF) in the splicing process in yeast. Our findings demonstrate that varying expression levels of this TF can influence the splicing of multiple genes producing isoforms with different degrees of intron retention, alternate 3’ splicing, alternate 5’ splicing, and exon skipping challenging our perceptions of the importance of alternative splicing in yeast.

1308T **Understanding the function of the C-terminus of Mms21 in genome stability** Cheung Li, Nkechinye Baadi, Yee Mon Thu Biology, Colby College

MMS21 and its human homolog NSMCE2 have various functions in DNA damage repair and response pathways, which play a vital role in defense against genetic diseases and disorders. Mechanistically, Mms21 functions as an E3 SUMO (small-ubiquitin like modifier) ligase which conjugates SUMO peptides to target proteins with the help of E1 and E2 SUMO ligases. We generated mms21Δ22 mutant in S. cerevisiae, a mutant in which the C-terminus is absent, to approximately mimic the human mutation found in a rare genetic disorder. We observed that a mutant of RAD5 (key regulator of the damage tolerance pathway) and the mms21Δ22 mutant exhibit a negative genetic interaction in the presence of methyl methanesulfonate (MMS), a DNA alkylating agent. This data suggests that Rad5 and the pathway regulated by the C-terminus of Mms21 operate in parallel to counteract MMS-induced DNA damage. Rad5 can function as an E3 ubiquitin ligase to initiate the template switching pathway. In addition, the helicase activity of Rad5 modulates fork regression when the replication fork encounters impediments. To further understand this intricate and essential network of DNA damage response and repair pathways, we will determine if the mms21Δ22 mutant genetically interacts with the rad5 helicase mutant or the rad5 E3 ubiquitin ligase mutant. In addition, we are performing an experiment to understand if the human MMS21 gene, NSMCE2, can functionally rescue the lack of yeast MMS21 gene using the plasmid shuffling assay. In this assay, wild type cells are first transformed with MMS21 on a high copy plasmid. This allows us to knock out the endogenous MMS21, which is essential for cellular survival. Cells with the episomal copy of MMS21 are then transformed with the plasmid carrying NSMCE2. Resulting cells with episomal copies of MMS21 and NSMCE2 will then be grown in the presence of a toxicant (5-FOA) that would force cells to lose the plasmid containing MMS21, while retaining the plasmid with NSMCE2. Growing mms21Δ cells with the episomal copy of MMS21 in 5-FOA led to lethality, confirming the feasibility of the approach. We are currently generating all appropriate strains to determine if the human copy can functionally complement the yeast gene. If such a system is successful, we will characterize cellular and molecular significance of additional NSMCE2 mutations associated with human genetic conditions

using this approach.

1309T **Transvection at MAL6 in yeast** Man Chang[1], Richard Needleman[2,2] [1]Lumicell, [2]retired

Homology-dependent gene expression, or transvection, is a poorly understood mechanism of gene expression. Studied most intensively in *Drosophila*, **we report here the first example of a natural yeast genetic system that exhibits both intra- and inter- chromosomal interactions that satisfies the definition of transvection.** First, specific deletions or deletion/insertions in *trans* silence an otherwise competent *MAL6* locus; second, deletions at *MAL6* on chr. VIII can affect expression of ectopic *MAL6* sequences on chr. V.

Notation: D*MAL63* refers to a large deletion of *MAL63* and replacement by *LEU2*(*mal63*). Δ*MAL61* refers to a large deletion of *MAL61* and replacement by *URA3*(*mal61*).

Position Effect at *MAL6*: The *MAL6* locus is telomeric complex with three active genes: *MAL63.* encoding a positive regulator, *MAL61,* encoding the maltose permease, and *MAL12* encoding maltase. The *MAL* genes are universally described as dominant: a MAL^+ x mal^- diploid is said to always be MAL^+. **However, this is not the case. The diploid *MAL63* Δ*MAL61/* D*MAL63 MAL61*, is completely maltose negative despite having a complete complement of *MAL* genes.** While this diploid is mal-, a diploid is also maltose negative when both deletions are in *trans (i.e. MAL63 MAL61*/**Δ***MAL63* **Δ***MAL61)*. The diploid *MAL6*/**Δ***MAL6,* where **Δ***MAL6* is the deletion of *MAL63 MAL61 MAL62* and their replacement by *LEU2*, is also negative, but the mitotic recombinants *MAL6*/*MAL6* are positive. Complementation occurs in both haploids and diploids between specific *MAL* genes integrated on chr. VII, but fails when they are present on chr. VIII**.**

Stated without proof: Non-complementation is not negative complementation due to subunit mixing, nor is it due to promoter interference or haploinsufficiency. Telomeric repression does not play a role in silencing. Transcription of *MAL63* in the mal- diploid *MAL63* Δ*MAL61*/D*MAL63 MAL61* is unaffected, but *MAL61* mRNA transcripts are undetectable.

Progress will require chromosomal association studies. Our observations do not reveal a clear mechanism-nor can purely genetic studies do so. Our hope is that the genetically simple *MAL6* system will here prove valuable. In respect to modelling the current situation, as Wittgenstein said, " Where one cannot speak, one must remain silent".

1310T **Why do transcription factors work together** Lindsey Snyder[1], Emily O'Brien[1], Jia Zhao[2], Thomas Cassier[1], Nicholas Schnicker[1], Bin He[1] [1]University of Iowa, [2]Massachusetts Institute of Technology

A prevalent feature in eukaryotic transcription is combinatorial regulation, or the requirement of two or more transcription factors (TFs) to co-activate a gene. This allows multiple upstream signals to be integrated and provides a way to tune specificity by requiring a defined motif configuration for activation to occur. A basic question, however, remains elusive: why do TFs need other TFs to function? And how does combinatorial regulation evolve? We take advantage of the natural variation in coTF dependence in the Phosphate Starvation (PHO) response system in two yeast species to study its genetic and biophysical basis. In S. cerevisiae, the main TF, Pho4 (ScPho4) relies on the coTF Pho2, whereas in C. glabrata, Pho4 (CgPho4) is largely independent of Pho2 and as a result activates 2-3 times more genes. We systematically tested >50 chimeras of ScPho4 and CgPho4 using a sensitive flow cytometry assay with a dual fluorescence reporter and identified the DNA binding domain (DBD), the putative Pho2-interaction domain, and a portion of the transactivation domain as collectively contributing to Pho2 dependence. We show that the CgPho4DBD binds ~4 times tighter than ScPho4DBD to the consensus motif in vitro with faster kinetics. A protein binding microarray revealed no difference in their binding specificity. CgPho4's transactivation domain has a higher activation potential than ScPho4 when assayed as a Gal4DBD fusion. We also show that CgPho4 is more capable of binding nucleosome-occluded DNA in vivo compared with ScPho4. Lastly, the two Pho4 orthologs differed significantly in their ability to interact and thus collaborate with Pho2. Our findings thus provide a detailed molecular picture of how co-TF dependence is mediated and how mutations in functional domains of the TF can lead to changes in codependency, which results in significant consequences in the regulatory output.

1311T **Reprogramming stress responses in yeast via mutation of mediator subunit Med8** Grace Kornegay, Kelsey P Brooks, William D Park Biochemistry & Biophysics, Texas A&M University

The Mediator complex plays key roles in gene expression in all eukaryotes and serves as a "central integrator" of genetic information. To serve this role, the Mediator must coordinate the expression of thousands of genes to support robust growth in a wide range of environmental conditions and coordinate appropriate responses to many different types of stress. However, the Mediator must also be able to evolve to meet the needs of diverse organisms. To determine whether we could change the way that the mediator integrates genetic information, we made a series of 17 mutants in which nine amino acid regions of Med8 were replaced with an unrelated sequence. Together, these cover $Med8_{20\text{-}173}$ and include all the regions of Med8 visible

in structure 5OQM of the Mediator complex from *S. cerevisiae,* with the exception of the Med18/20 tether region ($Med8_{190-223}$). When transformed into the standard yeast strain BY4742 by plasmid shuffling, all these mutants had only modest effects on the growth rate in rich media (YPD) at 30°C. However, four of the mutants strongly inhibited growth on YPD containing 6% ethanol. Two of these mutants were also sensitive to 1 M NaCl. Importantly, these mutants showed differential sensitivities to 6% ethanol, 1 M NaCl, as well as to heat stress (37°C). Additional mutants also differentially alter ethanol and methanol tolerance. Thus, these findings suggest that Mediator can be "re-programmed' through mutation to alter the expression of genes or gene sets in response to specific stimuli.

1312T **Identification of Enhancers Pertinent to Eye Development** Dwon A Jordana[1], Nathan Clark[2], Jason Presnell[3] [1]Biological Science, University of Pittsburgh, [2]Biological Sciences, University of Pittsburgh, [3]Human Genetics, University of Utah

Identifying conserved enhancers that function during ocular development is important, because it will signify regions sensitive to perturbations and possibly responsible for abnormal development. Discovering enhancers which regulate vital ocular genes helps to gain an understanding into the penetrance and severity of disease.

Regulatory regions pertinent to eye development will be detected by the presence of an amplified H3K27ac signature which is typically observed by enhancers. These regulatory regions will be located within a megabase window of ocular developmental genes such as Rx3 and Otx2b. I will use conservation within Teleosts in one dataset, but also deeper conservation across vertebrates to prioritize regions based on constraint to maintain these enhancers through time. Criteria for candidate regions are as followed: proximity to recognized genes, active enhancer histone marks (H3K27ac), open chromatin peaks in the optic vesicle at relevant time points, and absence of repressive histone modifications.

Beyond the study of sequence and genomic marks, I will establish necessity through Crispr Cas9 mediated deletions of the regions to determine how vital these regions are to development. In addition, I will test sufficiency by placing regions in an expression vector to determine if these putative enhancers alone are capable to drive expression of GFP in zebrafish. These experiments will demonstrate the value of these regions in regulating gene expression and possible relevance to disease.

I hypothesize that conservation across Teleosts will predict valuable regions with regulatory properties, and I will see greater functional impact for those regions that are conserved across all vertebrates.

1313T **The contributions of DNA accessibility and transcription factor occupancy to enhancer activity during cellular differentiation** Trevor E Long, Tapas Bhattacharyya, Madison Naylor, Andrea Repele, Shawn Krueger, Sunil Nooti Biology, University of North Dakota

During gene regulation, DNA accessibility is thought to limit the availability of transcription factor (TF) binding sites, while TFs can increase DNA accessibility to recruit additional factors that upregulate gene expression. Given this interplay, the causative regulatory events in the modulation of gene expression remain unknown for the vast majority of genes. We utilized deeply sequenced ATAC-Seq data and site-specific knock-in reporter genes to investigate the relationship between the binding-site resolution dynamics of DNA accessibility and the expression dynamics of the enhancers of *Cebpa* during macrophage-neutrophil differentiation. We found that, while the enhancers upregulate reporter expression during the earliest stages of differentiation, there is little corresponding increase in their total accessibility. Conversely, total accessibility peaks during the last stages of differentiation without any increase in enhancer activity. The accessibility of positions neighboring C/EBP-family TF binding sites, which indicates TF occupancy, does increase significantly during early differentiation, showing that the early upregulation of enhancer activity is driven by TF binding. Our results imply that a generalized increase in DNA accessibility is not sufficient, and binding by enhancer-specific TFs is necessary, for the upregulation of gene expression. Additionally, we show that high-coverage ATAC-Seq combined with time-series expression data can infer the sequence of regulatory events at binding-site resolution.

1314F **Genetic analysis of *lon-1, lon-2* and *sma-9*, three genes in the bone morphogenetic protein (BMP) signaling pathway in *C. elegans*** Sergio D Moreira-Antepara, Maria Victoria Serrano, Kelly (Jun) Liu Molecular Biology and Genetics, Cornell

Bone Morphogenetic Proteins (BMPs) are members of the TGFβ superfamily of signaling proteins and play critical roles in development and homeostasis across metazoans. Core BMP pathway components, including the ligand, the type I and type II receptors, and the cytoplasmic Smad proteins, are conserved in *C. elegans*. Null mutations in each of these core components result in a small body size phenotype. We are using double mutant analysis to determine the relationship between three additional BMP pathway components, LON-1, LON-2, and SMA-9. LON-1 is a secreted protein in the CAP superfamily, while LON-2 is a membrane anchored proteoglycan. SMA-9 is a zinc finger transcription factor that participates in the regulation of some BMP target genes. While mutations in either *lon-1* or *lon-2* result in a long body size phenotype, mutations in *sma-9* cause a small body size phenotype. Using existing null mutant alleles for *lon-2* and *sma-9*, as well as a null allele we

generated for *lon-1*, we measured the body lengths of different mutant worms at the same developmental stage (larval stage 4 or L4). We found that *lon-1(0); sma-9(0)* double mutants are as long as *lon-1(0)* single mutant worms, and that *lon-2(0) sma-9(0)* double mutants are as short as *sma-9(0)* single mutant worms. However, *lon-1(0); lon-2(0)* double mutants are longer than either single mutant. These results suggest that LON-2, SMA-9 and LON-1 do not function in a simple linear pathway. We are currently conducting additional experiments to further delineate the relationship between LON-1, LON-2 and SMA-9 in the BMP pathway and in body size regulation.

1315F **A Forward Genetic Screen in *Caenorhabditis elegans* Identifies Factors Regulating Transcriptional adaptation-like Ectopic Expression** Kuan-Lun Hsu, Vahan Serobyan, Didier Stainier Department of Developmental Genetics, Max Plank Institute for Heart and Lung Research

Transcriptional adaptation (TA) is a newly identified cellular response whereby an mRNA destabilizing lesion in one gene (mutant gene) causes transcriptional changes in other gene(s) (adapting gene(s)). In a previous study in *Caenorhabditis elegans*, we reported that an *act-3* (adapting gene) fluorescent reporter is expressed in the pharynx of wild-type worms, whereas in *act-5* (mutant gene) mutants the same reporter becomes ectopically expressed in the intestine. This observation indicates that TA can also lead to ectopic expression of the adapting gene, i.e. where the mutant gene is being expressed and its mRNA degraded. To identify the genetic basis and molecular mechanisms controlling the process whereby mutant mRNA cell-autonomously triggers TA, we performed a forward genetic screen. We identified several mutants exhibiting ectopic *act-3* reporter expression in the uterus, spermatheca, intestine, as well as body wall muscles. This diversity of expression patterns in the different mutants identified in our screen suggests that multiple genes regulate the expression pattern of this *act-3* reporter, and that the expression potential of this reporter, and potentially the *act-3* gene itself is not limited to a particular cell type. We are currently mapping these mutations to identify the loci involved in regulating the expression pattern of the *act-3* reporter. Mutants exhibiting transcriptional adaptation-like phenotypes will help in revealing the mechanisms underlying transcription adaptation.

1316F **The post-transcriptional regulation mechanism and functional importance of a key maternal mRNA, *glp-1*** Peren Coskun Biochemistry and Molecular Biotechnology, UMass Chan Medical School

Translational control of maternal mRNAs is a major form of gene regulation during germline development and embryogenesis. *C. elegans* Notch homolog *glp-1* is necessary for germ cell proliferation, and embryonic fate determination. The RNA binding proteins POS-1 and GLD-1 directly regulate the translation of GLP-1 protein by binding to specific elements within the *glp-1* 3' UTR. When POS-1 or GLD-1 binding is disrupted by mutation of their respective elements, the expression pattern of a *glp-1* 3' UTR reporter changes in both the germline and in embryos. The mechanism by which POS-1 and GLD-1 mediate translation repression is not well understood.

In this study, we show that wild-type *glp-1* transgenic reporter embryos have a shorter average polyA tails than transgenic reporters with mutated GLD-1 or POS-1 binding motifs. Mutation of these sites does not affect polyA site selection or the distribution of 3'UTR isoforms. We studied the effect of cytoplasmic polymerases, deadenylases and translation initiation factors on our transgenic reporters. Our results show that two cytoplasmic polyA polymerases, GLD-2 and GLD-4, have strikingly different effects on the expression of reporter transgenes harboring GLD-1 or POS-1 binding motif mutations. By contrast, none of the deadenylases affect the transgenic reporter expression. We also observed strong derepression of all reporters upon reduction of the translation initiation factor *ife-3*. The results reveal that POS-1 and GLD-1 exert their repressive effects in different ways through cytoplasmic polyA polymerase activity, while IFE-3 mediated translation repression is independent of both POS-1 and GLD-1. Lastly, we have examined the biological significance of *glp-1* 3'UTR to the worm reproduction by using CRISPR/Cas9 mutagenesis to generate *glp-1* 3'UTR mutations in the endogenous locus. Characterization of a 71 base pair mutation that deletes the GLD-1 and POS-1 binding sites in the *glp-1* 3'UTR reveals a 2-fold reduction in the number of embryos produced and a 4-fold reduction in the hatch rate. Imaging results show that the mutant embryos have patterning defects. Together, our results show that the *glp-1* 3'UTR contributes to reproductive health but is not essential to fertility.

1317F **RNAi-mediated regulation of *alg-3* and *alg-4* coordinates the spermatogenesis developmental program in *C. elegans*** Cara McCormick, Trilotma Sen, Alicia K. Rogers Department of Biology, University of Texas Arlington

Coordination of gene regulatory networks is necessary for proper execution of cellular programs throughout development. In *C. elegans*, spermatogenesis and oogenesis occur during different life stages (L4 and adult, respectively) within the hermaphroditic germline tissue. Yet, it remains unclear how these developmental programs are robustly executed, particularly during stressful conditions. Here we show evolutionarily conserved RNA interference (RNAi) pathways act to restrict expression of spermatogenesis genes to the L4 stage. We performed differential expression analysis of mRNA-seq and small

RNA-seq libraries from L4 and adult-stage wild-type and *mut-16* mutants, which lack a critical RNAi pathway protein, grown at permissive (20ºC) and elevated temperature (25ºC). Our analyses revealed spermatogenesis-enriched gene expression is developmentally mis-regulated in a small RNA-dependent manner at elevated temperature. Our findings indicate a novel RNAi-mediated regulatory cascade is essential for properly coordinating the spermatogenesis developmental program, particularly during heat stress. This is achieved through RNAi-mediated genetic switches that regulate the expression of the Argonautes, ALG-3 and ALG-4, to control ALG-3/4 pathway function. Disruption of this cascade results in severe spermiogenesis defects that underly sterility. This work provides key insights into the different molecular mechanisms RNAi pathways employ to maintain both oocytes and sperm cells' reproductive potential, and further highlights the complexities and importance of RNAi-mediated gene regulation in development.

1318F **Investigating how heterochromatin impacts aging and larval development in *C. elegans*** Arthur Colunga, Allison Sciarratta, Deepshikha Ananthaswamy, Teresa W Lee Biological Sciences, University of Massachusetts Lowell

Within the nucleus, genomes are organized and regulated by the modifications added to histone proteins. For example, di-methylation of histone H3 at its ninth lysine (H3K9me2) is a repressive modification associated with heterochromatin in *C. elegans*. Genomic levels of heterochromatin are regulated in part by two chromatin modifiers: JHDM-1 is a putative demethylase for H3K9me, while SET-32 is a methyltransferase for the newly discovered heterochromatin factor H3K23me. We have found that *jhdm-1* mutants experience a gradual increase in lifespan over many generations, and long-lived *jhdm-1* mutants have accumulated higher levels of H3K9me2. Others have shown that *set-32 (ok1457)* mutants are also long-lived, and have elevated levels of H3K9me3. Here, we explore whether the heterochromatin modifiers SET-32 and JHDM-1 may genetically interact. We find that *set-32; jhdm-1* double mutants experience a synergistic delay in larval development, with some individuals only reaching adulthood after ten days. We are currently using immunofluorescence to examine germline levels of heterochromatin factors, and assessing the epistatic interaction on lifespan. Taken together, these data will shed light on how heritable levels of heterochromatin can affect development and aging.

1319F **Mapping regulatory element evolution in nematodes** Thomas D King, Michael S Werner School of Biological Sciences, University of Utah

Enhancers and promoters appear to play different roles: promoters strongly activate transcription near the beginnings of genes, and enhancers impart spatial and temporal specificity to gene expression. However, both regulatory elements share important structural features, and both elements induce bidirectional transcription in many taxa, including nematodes. These similarities suggest that enhancers and promoters share a common evolutionary origin -- and that promoters could be reprogrammed into enhancers, or vice versa, over evolutionary time.

Our goal is to identify instances of these transitions, and identify and test the mechanisms by which they occur. We are mapping enhancers and promoters in several nematode taxa: *C. elegans* strain N2, *C. elegans* strain CB4856, *C. briggsae*, *C. tropicalis*, and *P. pacificus*. I have conducted internally calibrated ChIP (Ice-ChIP) to quantitatively map histone modifications characteristic of enhancers and promoters (H3K4me1, H3K4me3, and H3K27ac) in these taxa. I am also conducting capped small RNA sequencing in the same taxa to locate enhancers and promoters based on their characteristic bidirectional transcription patterns. Together, these datasets will provide a high-confidence annotation of enhancers and promoters across nematode taxa representing several different degrees of evolutionary divergence. I will identify examples where syntenic regulatory elements appear to have been reprogrammed, and look for correlations between these events and changes in histone modifications, DNA sequence changes, changes in expression patterns, and birth and death of nearby genes. I hope to gain insight into the mechanisms of regulatory element reprogramming, and I also hope to offer my regulatory element annotations as a resource for other researchers interested in the control and evolution of gene expression.

1320F **A new noncanonical biogenesis pathway generates a germline enriched miRNA family in C*. elegans*** Rima Sakhawala[1,2], Dustin Haskell[3], Anna Zinovyeva[3], Katherine McJunkin[4] [1]Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, [2]Johns Hopkins University, [3]Kansas State University, [4]National Institute of Diabetes and Digestive and Kidney Diseases

MicroRNAs (miRNAs) are short RNAs that post-transcriptionally regulate gene expression and play critical roles in development and differentiation. Despite their crucial biological importance, the biogenesis pathways that generate miRNAs are not completely understood. In canonical miRNA biogenesis, primary miRNAs are transcribed from intergenic loci or intronic regions by RNA polymerase II. These transcripts are then processed by the Microprocessor, an enzyme complex that consists of Drosha and RNA-binding protein DGCR8 (known as PASH-1 in *C. elegans*). Subsequent processing by Dicer produces the miRNA that is loaded into Argonaute to repress target mRNAs. However, rare noncanonical pathways also bypass the requirement for the Microprocessor and/or Dicer. Using a temperature-sensitive allele of *pash-1* in *C. elegans*, we discovered a unique family

of PASH-1-independent miRNAs, the *mir-1829* family. The *mir-1829* family resides in unusually long introns of three host genes that have no apparent overlapping functions. From 5' RACE data, we determined that the *mir-1829* family is derived from independent transcripts with a semiconserved TSS. We posited that these miRNAs are transcribed by RNA Polymerase III (RNAPIII) based on published RNAPIII ChIP-sequencing and conserved RNAPIII promoter elements. In support of this, CRISPR-mediated mutation of these elements abrogated *mir-1829b* expression. Furthermore, we have reintegrated a minimal transcriptional unit containing these putative RNAPIII promoter elements at an intergenic locus and observed expression of *mir-1829b* at similar levels to that of the endogenous locus. Downstream of transcription, although *mir-1829* family biogenesis bypasses Microprocessor, we have determined that it is Dicer-dependent by Northern blot and small RNA-sequencing. Thus, we have delineated a novel biogenesis pathway involving RNAPIII and Dicer, but not Microprocessor. Future work will examine the biology of these miRNAs. While our quadruple knockout of the miRNA family does not have an overt impact on viability or reproduction, we and others have demonstrated that it is enriched in a germline-specific Argonaute protein and may play a role in the robustness of the mitosis to meiosis transition in germ cell development.

1321F **Investigating HPV-E7 protein mediated disruption of the DREAM transcriptional repressor complex** Emily Washeleski[1], Ryleigh Parsons[2], Paul D Goetsch[2] [1]Michigan Technological University, [2]Biological Sciences, Michigan Technological University

E7, a small protein encoded by the Human Papilloma virus (HPV), plays a large role in the oncogenic progression of infected cells. Approximately 4.5% of new cancer cases worldwide develop from HPV infections. Although there is an available vaccine for the most common oncogenic HPVs, there remains no treatment to prevent oncogenesis if a patient does get infected. Oncogenic HPV-E7 proteins cause cancer via disrupting the transcriptional regulation of the cell cycle by inhibiting the function of the 3 mammalian pocket proteins, the Retinoblastoma protein (pRb), p107, and p130. Since HPV-E7 interacts with the pocket proteins through their "LxCxE" binding cleft, the viral oncoprotein impairs p107 and p130 from forming the DREAM transcriptional repressor complex as well as impairs pRb's tumor suppressor activity as a major checkpoint inhibitor of early cell cycle progression. The goal of this project is to better understand how the HPV-E7 oncoprotein disrupts DREAM complex function by establishing HPV-E7 transgenic lines in *Caenorhabditis elegans* using CRISPR/Cas9 mediated genome editing. Because the DREAM complex subunits are highly conserved, the sole *C. elegans* pocket protein homolog lin-35 is a likely candidate for binding by HPV-E7 proteins. Most HPV-E7 proteins, including all cancer associated strains, share a conserved LxCxE binding motif. This E7 motif is identical to the interaction motif required for LIN-52, another DREAM subunit, to bind to LIN-35 and form the *C. elegans* DREAM complex. I hypothesize that HPV-E7 proteins containing the LxCxE motif will compete against LIN-52 for association with the LIN-35 pocket protein, inhibiting DREAM complex formation and function. In contrast, I expect that HPV-E7 proteins lacking the motif will not disrupt DREAM formation. In *C. elegans*, loss of DREAM complex activity is readily observed via high penetrance of a synthetic multivulval (SynMuv) phenotype when combined with SynMuv A class gene mutants. I will use SynMuv phenotype analyses, along with co-immunoprecipitation, chromatin immunoprecipitation, and RNA-seq analyses, to determine the level by which transgenic HPV-E7 protein expression affects *C. elegans* DREAM function. Additionally, I will test if chemical antagonists to E7 LxCxE association will restore DREAM function in strains that generate a Muv phenotype. Overall, my goal is to establish a novel platform in *C. elegans* to study HPV-E7 protein specific associations *in vivo*.

1322F ***SPR-5; MET-2 maternal reprogramming cooperates with the Dream and MEC Complexes to regulate developmental cell fates*** Brandon S Carpenter[1], Jazmin S Dozier[1], Sandra S Nguyen[1], David Katz[2] [1]Kennesaw State University, [2]Emory University

At fertilization, histone methylation must undergo maternal reprogramming to reset the epigenetic landscape in the new zygote. During maternal reprogramming of histone methylation in the nematode, *C. elegans*, H3K4me is removed by the H3K4 demethylase, SPR-5, and H3K9me is subsequently added by the histone methyltransferase, MET-2. Recently, it was demonstrated that SPR-5; MET-2 maternal reprogramming antagonizes the H3K36 methyltransferase, MES-4, which maintains a transcriptional memory of a subset of germline genes between generations. Maternal loss of SPR-5 and MET-2 results in ectopic expression of MES-4 germline genes in somatic tissues and a severe developmental delay. Data from the Petrella and Ahringer Labs demonstrates that members of the DREAM Complex, a transcriptional repressor complex that regulates cell cycle, also represses germline genes in somatic tissues through H3K9me2 promoter marking. Furthermore, preliminary data from our lab shows that the histone deacetylation, MEC Complex, is also required to prevent a soma-to-germline transition. These data suggest that the DREAM Complex, MEC Complex, and SPR-5; MET-2 maternal reprogramming may work together to prevent ectopic expression of germline genes in somatic tissues and developmental delay. To test this hypothesis, we knocked down Dream Complex and MEC complex members in *spr-5; met-2* mutants using RNAi and found that knocking down either complex exacerbates the severe developmental delay that we normally observe in *spr-5; met-2* mutants leading to a complete L1 larval arrest. Using RNA-seq, we further demonstrate that knocking down Dream Complex and MEC complex members exacerbates the ectopic expression of MES-4 germline genes in *spr-5; met-2* mutant somas. Our findings provide mechanistic insight into how evolutionary conserved transcriptional repressor complexes and reprogramming of histone methylation

synergize to ensure proper germline versus somatic cell fates during development.

1323F **Inactivation of SPR-2, a histone acetyltransferase inhibitor, results in transgenerational germline mortality phenotype in *C. elegans*** Thalia Boston[1], Monica Reeves[2], Mackenzie S Roberson[1], Scott Roques[3], Karen L Schmeichel[1], David J Katz[2] [1]Biology, Oglethorpe University, [2]Cell Biology, Emory University, [3]Biological Sciences, University of Massachusetts

Work in *C. elegans by* Wen et al., showed that mutations in either *spr-5*, encoding an H3K4me2 demethylase, or *spr-2*, encoding a histone acetyltransferase inhibitor (INHAT), suppress the egg-laying defect of *sel-12* mutants by causing the normally germline-restricted paralog of *sel-12, hop-1,* to be expressed in the soma. Previously, we found that *spr-5* mutants in *C. elegans* exhibit transgenerational germline mortality, where the brood size of the population steadily declines across generations. In these animals, transcriptionally activating H3K4me2 marks accumulate in germline genes leading to the aberrant expression of these genes in the soma over time. Like H3K4me2, histone acetylation is associated with transcriptionally active chromatin. Therefore, by inhibiting the accumulation of histone acetylation in the germline, SPR-2 may also function to prevent germline genes from being inappropriately activated in the soma of the subsequent generation. To determine whether *spr-2* mutants exhibit a germline mortality phenotype, we examined the brood size of homozygous mutant animals harboring one of two *spr-2* mutant alleles: *spr-2(ar211)*, with a nonsense mutation in *spr-2*'s third exon, and *spr-2(ar199),* with a deletion in the gene's promoter. Over 16 generations, *spr-2(ar211*) point mutant worms yielded brood counts that were comparable to those seen in wild-type N2 worms (average brood count was 278.3, $p>0.05$). However, promoter-deleted *spr-2(ar199)* mutants displayed a statistically significant reduction in brood size starting at F7 (average brood count was 49% of N2, $p < 0.05$). By generation F13, populations were severely infertile exhibiting brood counts that were 10% of N2 ($p<0.05$). The germline mortality of *spr-2(ar199)* animals raises the possibility that histone acetylation may be accumulating transgenerationally, resulting in the expression of germline genes in the soma. We are currently performing experiments to test this possibility. As far as we know, there is no previous evidence that histone acetylation can function as a transgenerational transcriptional memory.

1324F **Guiding mRNA to nuclear periphery: Lessons from imb-2 in C elegans** Ambika Basu, Lindsay Winkenbach, Dylan Parker, Erin Nishimura Biochemistry and Moelcular Biology, Colorado State University

The *Caenorhabditis elegans* maternally inherited mRNA transcript *imb*-2(Importin beta-2) concentrates at the nuclear periphery along with its encoded protein. To understand signals that guide the *imb-2* transcript to the nuclear periphery, we assessed whether its mRNA sequence or peptide sequence direct localization. To test the sufficiency of the 3'UTR of *imb-2* in driving localization, the 3'UTR was appended to a naive reporter. The reporter could not make it to its desired location, hence we concluded that 3'UTR was not sufficient. Experiments with translation-inhibitors cycloheximide and puromycin revealed that ongoing translation elongation is not necessary. Instead, an intact ribosome nascent chain complex is required for *imb-2* localization. This suggests that peptide sequences in the nascent IMB-2 protein direct nuclear localization of the nascent chain, the ribosome, and the associated *imb-2* mRNA. We went on to ask if the mRNA coding sequence or amino acid sequence is necessary for mRNA localization. For this, we re-coded the mRNA coding sequence while preserving a synonymous IMB-2 protein sequence intact by virtue of redundant codon usage. We observed that the re-coded mRNA led to loss of mRNA and protein localization and also caused mRNA instability. This brought us to the conclusion that the peptide sequence alone does not recapitulate the wild-type mRNA behavior. Taken together, *imb-2* nuclear periphery localization depends on a combination of mRNA and amino acid encoded information to ensure mRNA stability, translational complex formation, and transport.

1325F **Histone methylation readers *cec-3* and *cec-6* regulate germ granule integrity and small RNA pathways** Tammy L Lee, Chengyin Li, Phoebe AW Bhagoutie, Arneet L Saltzman University of Toronto

Small RNA and chromatin regulation pathways cooperate to safeguard germline immortality and the inheritance of genetic information. Importantly, many factors involved in small RNA-mediated gene regulation reside in evolutionarily conserved perinuclear germ granules. We previously found that two *C. elegans* chromodomain proteins, *C. elegans* Chromodomain (CEC)-3 and CEC-6 recognize heterochromatin-associated histone H3K9 and H3K27 methylation *in vitro*. *cec-3; cec-6* mutant animals show a progressive sterility or 'mortal germline' phenotype, but the underlying cause of this fertility defect is unknown. Live imaging analysis of GFP-tagged germ granule components PGL-1 and DEPS-1 reveals that germ granule formation is disrupted in *cec-3; cec-6* mutant adult germlines. Furthermore, progressive loss of germ granule integrity was accompanied by defects in meiotic prophase progression as *cec-3; cec-6* lines approach sterility. Our small RNA-seq analysis also shows that late generation *cec-3;cec-6* mutants have increased antisense small RNAs targeting a germline reporter that remained silenced for extended generations, and differentially regulated WAGO-class small RNAs. Our transcriptome analysis suggests that a subset of repetitive elements, transposons, germ granule components, and endogenous siRNA targets are mis-regulated in *cec-3; cec-6* mutants. While *cec-3; cec-6* mutants have similar germ granule and mortal germline phenotypes to the piRNA Argonaute

mutant *prg-1,* our analyses using a germline piRNA sensor suggest that *cec-3* and *cec-6* are not required for piRNA-mediated silencing. We are currently investigating how *cec-3* and *cec-6* impact the small RNA repertoire and transcriptome over generations. Together, our results suggest a role for these heterochromatin reader proteins in the small RNA and chromatin silencing pathways that maintain germ cell fate and genome integrity.

1326F **Control of spermatogenesis developmental program via RNAi-mediated regulation of ALG-3/4 small RNA pathway in *C. elegans*** Trilotma Sen, Cara McCormick, Alicia K Rogers Biology, The University of Texas at Arlington

Gene regulation is necessary for maintaining coordination of molecular mechanisms during development to ensure proper fertility. Precise activation and silencing of genes within totipotent germ cells is crucial for gametogenesis. In *C. elegans,* RNA interference (RNAi) pathways restrict spermatogenesis during the L4 stage and permit the expression of oogenesis genes in the adult stage. Yet, it remains unclear how these developmental programs are robustly executed, particularly during stressful conditions. We leveraged *mut-16* mutants, which exhibit sperm-based infertility at elevated temperature (25ºC), to examine how RNAi pathways protect sperm reproductive potential during stress. We found that sperm genes were developmentally mis-coordinated in heat-stressed *mut-16* mutant hermaphrodites. Bioinformatic analyses revealed the mis-regulated genes largely overlapped with ALG-3/4 pathway targets indicating that small RNA mediated regulation from the *mutator* complex is essential throughout development, despite ALG-3/4 pathway being independent of the *mutator* complex for amplification of its downstream 22G-RNAs. Further analysis revealed developmental mis-regulation of *alg-3* and *alg-4* gene expression in heat-stressed *mut-16* mutants, correlating with changes in 22G-RNA and 26G-RNA levels at the *alg-3* and *alg-4* genomic loci. These discoveries suggest that small RNA pathways play a role in activating and deactivating the ALG-3/4 pathway at the proper developmental stages.

1327F **Genome-wide studies of natural variation reveal putative members of the Gap Gene Regulatory Network** Lossie (Elle) Rooney[1], Gregory T. Reeves[2] [1]Biological Sciences (Genetics), North Carolina State University, [2]Texas A&M University

Axial patterning conveys positional information throughout the body of an organism during embryogenesis through a cascade of gene expression controlled by a gene regulatory network (GRN). GRNs contain multiple agents, including transcription factors and genomic sequences, that interact to determine complex traits. Determination of these traits may be impacted by perturbation of part of the network unless network architecture effectively adapts to maintain a robust phenotype. Across a population, natural genetic variation is one such perturbation. Here, we use the natural variation found in the *Drosophila melanogaster* Genetic Reference Panel (DGRP) to examine the Gap GRN that patterns the anterior-posterior (AP) axis of the early (1-3 h old) *Drosophila* embryo. AP axis specification is a well-studied phenomenon of early embryogenesis for which many genes, regulatory sequences, and transcription factors have been identified, though the knowledge of its functioning remains incomplete. The DGRP is a panel developed for association mapping while maintaining the natural variation of the original population and showing between-line variability for many phenotypes. Our lab has previously shown differences in spatial expression patterns of known Gap GRN genes using a small subset of DGRP lines. We expand on these findings with additional lines of the DGRP, using RNAseq as well as confocal microscopy, and discuss targets for further study.

1328F **Determining Jagunal as a Regulator in the Development of the Drosophila larval brain** Stephanie Uzordinma Awuzie[1], Judy Abuel[2], Maria Mendoza[1], Todd Nystul[3], Blake Riggs[2] [1]San Francisco State University, [2]Cell and Molecular Biology, San Francisco State University, [3]Anatomy, University of California, San Francisco

Asymmetric cell division is a highly conserved process across all species that is necessary for the proliferation of different cell types. This process is facilitated by the unequal partitioning of apical and basal cell fate determinants during stem cell division. Although asymmetric cell division has been extensively studied, the mechanism driving the localization of these cell fate determinants to generate cell differentiation is less well understood. Jagunal is a highly conserved integral transmembrane Endoplasmic Reticulum protein that is believed to play a role in asymmetric cell division. Here, we use immunohistochemistry and confocal microscopy to understand Jagunal's role in regulating the neural cellular composition of Drosophila during early development. This experimental approach has revealed that deficiencies in Jagunal result in an increase of neuroblasts compared to differentiated neurons within the larval brain. It has also been observed that Jagunal mutant brains display a mislocalization of basal cell fate determinants, suggesting that Jagunal is a key player in maintaining a balance of self-renewal and cell differentiation during the early brain development of Drosophila. To further study this, a single-cell transcriptome atlas will be built by sequencing the central nervous system across Jagunal deficiency to further examine Jagunal's downstream impact on cell heterogeneity, and gene expression. Understanding the mechanism of cell differentiation helps us to understand the role of JAGN1, the human homolog of Jagunal, in diseases linked to the disruption of cell differentiation, such as neutropenia; an immunocompromising blood condition.

1329F **Mapping the relationship between transcription factor concentration and gene expression using scanning Fluorescence Correlation Spectroscopy** Sadia Siddika Dima, Gregory T. Reeves Chemical Engineering, Texas A&M University

Gene regulation is a crucial biological process in development, homeostasis, and disease. Despite the pivotal role of transcription factors (TFs) in controlling gene expression, mechanisms such as how DNA becomes accessible to the TFs for binding and the map between TF concentration and gene expression are still not well understood. Therefore, in this work, we measured the absolute concentrations of multiple cooperative factors in the *Drosophila* blastoderm embryo over a 1.5 h period and correlated these concentrations to gene activation.

During the earliest stages of embryogenesis, the zygotic genome in most metazoans, including the fruit fly, remains transcriptionally silent after fertilization. Transcriptional activation of the zygotic genome coordinated with the degradation of maternally provided mRNAs occurs hours after fertilization, at a period termed as maternal-to-zygotic transition. In *Drosophila melanogaster*, the pioneer factor Zelda (Zld) acts as a master regulator for the transition and transcriptional activation of the zygotic genome. Unlike most other TFs, Zld can bind to the DNA in the context of nucleosomes and makes the chromatin accessible to other TFs thus helping early gene expression. Zld has been found to facilitate the binding of the dorsal-ventral patterning factor Dorsal (Dl) and the anterior-posterior patterning factor Bicoid (Bcd). Quantitative measurements of the biophysical parameters such as the nuclear concentration of the factors and the fraction bound to DNA as well as the change of these parameters over time are required for a better understanding of the system in which Zld helps these TFs bind to their motifs. Therefore, we performed Raster Image Correlation Spectroscopy (RICS), a type of scanning Fluorescence Correlation Spectroscopy, on images of live embryos between 1 and 3 h old carrying the factors tagged with GFP and histone-RFP marker to extract information about molecular dynamics and concentrations. From the results, we hypothesize that in early nuclear cycles (ncs), higher concentrations of Zld bound to DNA help the binding of Bcd and Dl, which are present in lower nuclear concentrations. In the later ncs, Zld binding attenuates, and other pioneer-like factors as well as higher concentrations of Bcd and Dl assist the process. The disagreement of our results with standard equilibrium models of TF/DNA interactions suggests that the ability of Zld to bind the DNA changes over time.

1330F ***Cis*-element bypass (redundancy) in *Drosophila melanogaster*** Anthony Percival-Smith Biology, Univ Western Ontario

Phenotypic specificity of transcription factor function was often presumed. For example, Proboscipedia was presumed to be specifically required for maxillary palp and proboscis determination, and Ultrabithorax was presumed to be specifically required for haltere determination. Testing whether transcription factor dependent phenotypes were rescued by TFs other than the TF resident to the *TF* locus, that is non-resident TFs, uncovered extensive redundancy of TF function (phenotypic nonspecificity) challenging the presumption of phenotypic specificity of transcription factor function. The frequent rescue of seven of eight transcription factor (TF) dependent phenotypes by multiple, non-resident TFs that recognize distinct DNA binding sites indicates extensive redundancy of TF function in developmental/behavioral pathways that is not based on recognition of similar DNA binding sites. Because distinct DNA-binding sites are recognized in phenotypic nonspecificity, there is the hypothetical expectation that the *cis*-regulatory elements used by non-resident TFs in a regulatory region are different from those used by the resident TF; simply put, the redundancy of trans-acting factors should be mirrored in the redundancy of *cis*-acting regulatory sequences. The rescue of depigmentation of the *dsx* null mutant abdomen by the expression of Doublesex female (DSX^F) is also rescued by the expression of Bric a Brac 1 (BAB1), Antennapedia (ANTP), Eyeless (EY) or Oddskipped (ODD). Analysis of epistasis suggests that all four TFs are epistatic to DSX^F and that ANTP, EY, ODD are epistatic to *bab* suggesting that these three TFs act after BAB or substitute for BAB function. BAB represses Yellow (Y) expression during the patterning of female abdominal pigmentation by binding to regulatory sequence in the *y* regulatory region. The *cis*-elements required for BAB regulation of *y* expression are well-characterized; therefore, the *y* gene may provide a platform for the analysis of the requirement of *cis*-regulatory elements for phenotypic nonspecificity. Using *y* regulatory elements that have BAB binding sites and that lack BAB binding sites, I am testing whether the TFs ANTP, EY and ODD require the BAB binding sites or not for repression of *y* expression. The non-requirement of the *cis*-acting BAB binding sites of *y* (*cis*-element bypass) would suggest that other DNA binding sites in the regulatory sequence are being used to repress *y* expression indicating *cis*-element redundancy.

1331F **Interrogating mitochondria-nuclear crosstalk: A novel perspective to Single nuclei RNA Sequencing** Snigdha Gupta[1], Fan Zhang[2], Hong Xu[2] [1]Laboratory of Molecular Genetics, National Heart Lung and Blood Institute, NIH, [2]National Heart Lung and Blood Institute, NIH

Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are a genetically and clinically heterogeneous group of autosomal recessive disorders representative of a severe reduction in mtDNA content. MDS arise due to defects in mtDNA maintenance caused by mutations/dysregulation **in** nuclear gene that function in either mitochondrial nucleotide regulation or mtDNA replication. We aim to identify novel pathways/candidate genes involved/associated with mitochondrial DNA depletion/ mitochondrial defects and thus contribute to various disease pathologies. We utilize an Auxin -inducible UAS/Gal4 system to express a mitochondrial targeted restriction enzyme to deplete mtDNA in a spatio-temporal manner and conduct single nuclear RNA sequencing to study the mito-nuclear crosstalk. We generated mtDNA deficiency in fly eyes at two developmental

stages: the larval eye disc that is mainly consisted of mitotic cells and adult eyes that contain terminal differentiated photoreceptor neurons. The nuclei in larval eye discs and adult eyes were marked with Lamin-gfp and isolated using FACS base sorting. The GFP positive nuclei from the control and mtDNA depleted samples were subsequently sequenced using 10x genomics. Data analyses are currently underway. We will expand our study in other energy demand tissues including muscle cells and heart, which are also frequently affected by mitochondrial DNA depletion. These analyses will allow to detect tissue specific candidates/pathways involved in pathophysiological processes of mtDNA depletion and investigate possible mtDNA and nuclear genome interactions responsible for tissue homeostasis and maintenance.

1332F **Genetic and bioinformatic analyses reveal novel transcriptional regulation of mitochondrial ETC biogenesis in *Drosophila*** Fan Zhang, Anne Lee, Anna Freitas, Zongheng Wang, Snigdha Gupta, Zhe Chen, Hong Xu NHLBI, National Institutes of Health

Mitochondria carry out energy metabolism and modulate various cellular signaling pathways, and hence are crucial for tissue development and homeostasis. Mitochondrial mass and respiration are tightly regulated in cope with the tissue and development- specific metabolic demands. Mitochondrial genome encodes handful genes of respiratory chain complexes, whereas most mitochondrial proteins are encoded on the nuclear genome. However, the mechanisms coordinating these two genomes in the regulation of mitochondrial biogenesis remain largely unknown. To identify transcription circuits involved in such regulation, we performed a candidate RNAi screen covering 638 transcription factors annotated in the fly genome. We reasoned that impaired mitochondrial biogenesis would synergistically interact with mtDNA deficiency in disrupting tissue development. By combining individual RNAi with mtDNA deficiency, we identified 77 transcription factors that may be involved in the transcriptional regulation of mitochondrial biogenesis and mtDNA genome. Additional genetic and genomic analyses comfired the pivotal role of a novel transcription factor, CG1603, and its upstream factor YL-1 in mitochondrial biogenesis. Moreover, we constructed a regulator network of positive hits using the published CHIP-seq data. The network analysis revealed extensive connections and complex hierarchical organization underlying the transcription regulation of mitochondrial biogenesis.

1333F **Decoding the regulatory role of CrebA in secretory output during salivary gland development in Drosophila melanogaster** Dorian M Jackson[1], Dan Peng[1], Matthew Slattery[2], Patrick Cahan[3], Deborah Andrew[1] [1]Cell Biology, Johns Hopkins University, [2]Biomedical Sciences, University of Minnesota, [3]Biomedical Engineering, Johns Hopkins University

Whereas secretion occurs in all cells of higher organisms, there are specialized organs uniquely dedicated to secretion, including, for example the human pancreas, mammary, lacrimal, and salivary glands. Although essential for organismal well-being, the early regulatory networks and developmental events that define their capacity to function as secretory organs are less understood. The Drosophila salivary gland (SG) – a relatively simple dedicated secretory organ – is a premier model for elucidating how high-capacity secretory function is achieved. Studies in the last ~20 years have revealed key transcription factors (TFs) controlling both secretory capacity and secretory specificity. The CrebA leucine zipper TF has been shown to coordinate increases in secretory capacity in the SG as well as in other professional secretory cells of the Drosophila embryo. Loss of CrebA results in a reduction in secreted SG lumen volume and structural defects in the cuticle secreted by epidermal cells that correspond to significant decreases in the levels of expression of the secretory pathway component genes (SPCGs) that encode the protein machinery of secretory organelles. CrebA boosts secretory capacity through direct binding and transcriptional activation of SPCGs and of other genes involved in secretion. Whereas binding of CrebA is essential for direct regulation of gene expression, binding and regulation of specific genes are not tightly correlated. A previous study showed that a subset of genes that are directly bound by CrebA had no overt changes in their expression in the absence of CrebA; indeed, studies to date suggest that CrebA binds far more genes in the SG than are affected by its loss or overexpression. Thus, we aim to discover the features of CrebA binding that distinguish genes both bound and affected by CrebA from the features of CrebA binding in genes unaffected by CrebA loss or overexpression. We have done single cell RNA sequencing (scRNA-seq) to identify the full set of SG genes whose expression change during embryonic development and chromatin-immunoprecipitation and sequencing (ChIP-seq) to identify the full set of SG genes bound by CrebA during this same period. Using computational approaches, we are now searching for correlations in binding parameters to changes in expression levels in WT versus CrebA null samples to build and test models of CrebA-dependent gene expression in the SG.

1334F **Elucidating the roles of ecdysone signaling in adult *Drosophila melanogaster*** Katherine Fhu[1], Zachary Drum[2], Joseph Coolon[3] [1]Biology, Wesleyan University, [2]University of North Carolina at Chapel Hill, [3]Wesleyan University

Ecdysone is a steroid hormone found in arthropods that is vital in coordinating life cycle transitions in *Drosophila* and other insects. Modulation of 20-hydroxyecdysone (20E) levels, the active form of ecdysone, is crucial to initiate larval molting, pupariation, and metamorphosis events. While ecdysone's role in *Drosophila* development is well studied, much less is known about the presence and function of ecdysone in adults. Previous work has revealed that ecdysone affects various processes

in adult *Drosophila*, including reproduction, lifespan, and stress responses. Here, we seek to further understand the roles of ecdysone by observing genome-wide gene expression changes in response to perturbing ecdysone signaling in fully developed flies. This was achieved in two different experiments: 1) feeding adult females excess 20E and 2) expressing a dominant-negative mutant form of the ecdysone receptor (EcR). RNA-sequencing was performed on both sets of experiments, yielding sets of significantly differentially expressed genes associated with perturbation in ecdysone abundance. These data will identify new candidate target genes of ecdysone signaling in adult *Drosophila*, further distinguishing biological and molecular processes that are affected by this essential hormone in different life stages of insects.

1335F **A proximity labeling approach to discover novel members of the Drosophila histone locus body** Casey A Schmidt[1], Leila E Rieder[2] [1]Biology, Emory University, [2]Emory University

Histone biogenesis is strictly controlled in cells. Reduced histone expression leads to developmental arrest, and excess histone production is toxic to cells; thus, there are precise mechanisms in place to ensure that histones are manufactured at the appropriate time and in the correct amount. To achieve these regulatory requirements, the Drosophila melanogaster replication-dependent histone genes are arranged in a 5kb array that is tandemly repeated over 100 times at a single genomic locus. Transcription and processing factors concentrate in a nuclear body known as the histone locus body (HLB), which forms specifically at the locus. Historically, HLB factors have been largely discovered by chance, and few are known to directly interact with DNA. It is therefore unclear how the HLB identifies and targets the histone gene locus. To expand the list of known HLB members, we are using an in vivo proximity labeling approach. We targeted the promiscuous biotin ligase TurboID to the histone locus and will identify biotinylated proteins by mass spectrometry. Our preliminary experiments show robust and specific biotinylation coincident with Mxc, which is found exclusively in the HLB. This screen will be the first to address HLB composition in the context of development. Our approach, together with our previous candidate-based bioinformatics screen, will reveal novel HLB members, allowing us to further elucidate the unique mechanism of histone gene regulation.

1336F **Amino Acid Transporter Expression Screen Across Distinct Tissues in *Drosophila melanogaster* Adults** Ymani Wright, Alissa Armstrong Biological Sciences, University of South Carolina

Dietary amino acids play important roles in metabolic processes that support normal tissue growth, repair, and development. Amino acid transporters facilitate migration of amino acids into the cell. In *Drosophila*, we have previously shown that amino acid transporter knockdown in adult adipocytes mediates ovarian responses to dietary protein input. The Drosophila genome encodes 51 genes thought to be amino acid transporters and high-throughput studies suggest differential tissue expression. We hypothesize that distinct tissues including the fat body, ovary, brain, muscle, and gut require distinct dietary protein inputs to mediate their normal physiological functions. Thus, we first set out to determine the complement of amino acid transporters expressed by several tissues using an RT-PCR screen of 26 solute carrier amino acid transporters. We find that expression of the 26 amino acid transporters tested varies between these distinct tissues. For example, the ovary expresses low levels while the brain expressed high levels of *Aralar1*. Currently, we are determining if an organism's physiological state influences amino acid transporter expression by measuring amino acid transporter transcript and protein levels in tissues across different ages, diets, and sexes. In future studies, we will focus on determining amino acid transporter expression under obesogenic conditions. The genetic screen of amino acid transporter expression will help elucidate specific amino acid requirements for distinct tissues under D. melanogaster homeostatic and non-homeostatic conditions.

1337F **Investigating the Role of Histone Chaperones in Coordinating H3 and H3.3 Function in *Drosophila melanogaster*** Lucy C Grossmann[1], Jeanne-Marie E McPherson[2], Daniel J McKay[2], Robert J Duronio[1] [1]Biology, University of North Carolina at Chapel Hill, [2]Genetics, University of North Carolina at Chapel Hill

Histones are required for packaging DNA and regulating genome function. Controlling the abundance of histones within cells is essential, as too many or too few histones disrupts development. Eukaryotic genomes encode two types of histones: canonical and variant. Canonical histones are expressed only during S-phase, whereas variant histones are expressed throughout the cell cycle. We propose that coordination between canonical and variant histones contributes to achieving proper histone abundance. Canonical histone *H3.2* and variant histone *H3.3* differ by four amino acids, three of which modulate their interactions with chaperone complexes that deposit these histones into chromatin. Through an unbiased screen, we identified a genetic interaction between histone *H3* gene copy number and *Yem*, an H3.3-specific chaperone. This finding suggests that histone chaperones play a critical role in modulating the relative levels of H3.2 and H3.3 in cells. Since Yem and other chaperones bind histones in a type-specific manner, we hypothesized that they contribute to the coordination of canonical and variant histone abundance in cells. To this end, we have generated mutants that produce the canonical H3.2 protein from the *H3.3* genes (*H3.3*$^{H3.2}$), and thus only produce H3.2 protein outside of S-phase. Our results suggest that H3.3$^{H3.2}$ is incorporated into chromatin outside of replication. To determine which histone H3 chaperones are interacting with and depositing H3.3$^{H3.2}$ into chromatin we are performing genetic interaction experiments using histone chaperone

mutations and *H3.3*$^{H3.2}$ mutant genotypes. In our initial set of experiments, we are focusing on the H3.3-specific chaperone genes *Yem*, *HIRA*, and *Daxx* and the H3.2-specific chaperone gene *Caf-1*. We anticipate these experiments will provide insight into mechanisms by which cells coordinate histone type and abundance to regulate genome function.

1338F **Contextual cures drive locus specific function of a context-dependent transcription factor** Lauren J Hodkinson[1], Julia Gross[2], Casey A Schmidt[1], Pamela P. Diaz-Saldana[2], Leila E Rieder[1] [1]Department of Biology, Emory University, [2]Emory University

Despite binding similar *cis* elements, transcription factors (TFs) can perform context-dependent functions at different genomic locations. We broadly aim to understand how TFs integrate *cis* sequence and genomic context to function uniquely at different loci. One example of a context-specific TF in the genetic model *Drosophila* is Chromatin-Linked Adapter for MSL Proteins (CLAMP), which targets similar GA-rich *cis* elements on the X-chromosome and at the histone locus but recruits very different, locus-specific TFs. On the male X-chromosome, CLAMP recruits dosage compensation factors however, at the histone locus, CLAMP promotes formation of the conserved histone locus body (HLB) to regulate expression of the replication-dependent histone genes. Here we investigate how *cis* element origin can impact CLAMP function in the context of the histone genes. We engineered flies to carry a transgenic histone array in which we replaced the natural CLAMP-recruiting GA-repeat *cis* elements in the bidirectional promoter of histone genes 3 and 4 (*H3H4*p) with CLAMP-recruiting GA-rich elements from the X-chromosome. We assessed how X-linked *cis* elements impact CLAMP function by staining third instar larval polytene chromosomes with antibodies to a core HLB protein and an X-chromosome specific factor. When we replaced the *H3H4*p with an X-linked CLAMP recruiting region, HLB factors were not recruited but an X-chromosome factor was recruited to the transgene. However, when we replaced only the natural GA-repeats with GA-rich regions originating from the X-chromosome, either the HLB factor or the X-chromosome factor was recruited to the transgene. Our observations indicate that CLAMP incorporates both sequence and local contextual cues to dictate its function.

1339F **Investigation of Distinct Adipose Tissue Classes in Adult *Drosophila melanogaster*** Isaiah H Williams, Alissa Armstrong Biological Sciences, University of South Carolina

The energy storage and thermoregulatory roles of distinct classes of fat tissue, white, brown, and beige fat, have been well characterized. However, we are just beginning to understand how the fat tissue mediates its endocrine role. In *Drosophila melanogaster*, the adipose tissue, or fat body, uses nutrient sensing pathways to relay nutritional information to the diet-responsive ovary. To determine if there are distinct adipose tissue classes that mediate inter-organ communication in fruit flies, we are characterizing the morphological and functional regionalization of the adult fat body. Using the Gal4/UAS system, our current goal is to generate a comprehensive expression atlas of fat body-Gal4 transgenic fly lines that will then be used to investigate functional regionalization. We are examining reporter gene expression in 42 driver lines in the fat body in the head, thorax, and abdomen. Additionally, we are assessing tissue specificity by determining driver expression in the gut, ovary/testis, brain, and muscle. Thus far, we have examined 16 lines in the adult and larval fat body. For some driver lines, we find previously unreported driver expression. We also observe expression differences relative to age and level. After establishing expression patterns for these driver lines, we will investigate how diet affects fat body driver expression to determine how nutritional input impacts cellular and molecular mechanisms of adipose tissue function. In future studies, we will use drivers that correlate with specific adipose tissue subtypes to genetically ablate adipocytes and assess effects on inter-organ communication, particularly fat-to-ovary. The characterization of the diverse expression patterns in the fat body will aid driver selection for more precise control in *Drosophila* adipose tissue studies.

1340F **The role of chromatin-binding proteins on the trans-regulatory impact of variation in Y-linked heterochromatin** Shane F Warland, Keegan Kelsey, Elissa J. Cosgrove, Andrew G Clark Genetics, Cornell University

In most higher order eukaryotic organisms, a substantial portion of the genome is composed of large quantities of repetitive DNA, often in the form of short tandem repeats known as satellites. These satellite repeats play essential roles in centromeres and telomeres, but despite this, satellites are among the fastest evolving regions of the genome. The high rate of turnover of satellites is accompanied by high levels of inter-individual variation in copy numbers. The Y chromosome of *D. melanogaster* consists of nearly 40 Mb of satellite repeats, and its high level of variation in repeat copy number makes it an ideal natural perturbation of the dosage of satellites in the genome. From our Global Diversity Lines of *D. melanogaster*, we derived a series of isogeneic fly lines which only differ by their geographically disparate Y chromosome. Analysis of these lines has revealed two key observations. First, that heterochromatic repeat content varies remarkably among lines, leading to more than 3-fold variation in total Y-linked satellite content resulting in over 10 Mbp of sequence lost or gained across the lines. Second, RNA-seq analysis shows differential expression of hundreds of genes, with some gene transcripts correlated with distinct satellites. Correlative evidence is consistent with individual satellites binding with some specificity to particular transcription factors, allowing the expansion and contraction of specific satellite repeats to fine-tune individual protein levels in the genome. We applied CUT&RUN to these lines to quantify binding of the pioneer factor GAF, which has been shown

to bind to AAGAG and other AG-rich repeats, and find significant differences among Y-replacement lines in local binding, correlated with Y-linked copy number of relevant satellites. The degree of specificity of repeat copy number, impacting availability of chromatin-binding proteins and concordant impact on gene expression is being explored through a series of targeted CUT&RUN experiments. Our results present a promising mechanism for the "heterochromatin sink," adding a specificity of the connection between repeat abundance, binding protein availability and target gene expression modulation. This view of the Y chromosome as a trans-regulator of the rest of the genome has implications for the evolutionary significance of the Y chromosome and for heterochromatin in general.

1341F **Transcriptional Regulation of Stochastic Cell Fate Specification in the *Drosophila* eye** Emma Steinson[1], Rina Helt[1], Robert John Johnston[1], Elizabeth Urban[2], Lukas Voortman[2] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

During development, cell fate decisions are often driven by lineage and signaling mechanisms. Generally, these mechanisms are robust, overcoming molecular noise to generate stereotypical patterns of cells. However, in some cell populations, stochastic cell fate specification harnesses molecular noise to diversify cell types to produce distinct proportions, but random patterns of cell types within tissues. Though stochastic cell fate specification is vital during development, the mechanisms that regulate these fate choices are poorly understood. Here, we investigate how cell-intrinsic mechanisms, specifically transcription and chromatin regulation, contribute to stochastic cell fate choices in the developing *Drosophila* eye. In the fly retina, R7 photoreceptors make a random, binary choice to express Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). The random pattern of R7 subtypes is controlled by the stochastic ON/OFF expression of a transcription factor called Spineless (Ss): Ss^{ON} R7s express Rh4 and Ss^{OFF} R7s express Rh3 in the adult retina. Although each R7 randomly chooses subtype fate, 67% choose Ss^{ON} R7 fate and 33% choose Ss^{OFF} R7 fate, generating consistent ratios of cell types yet random patterns. Our data suggest that stochastic expression of *ss* is controlled by the dynamic regulation of transcription and chromatin during development. An *early enhancer* activates expression of *ss,* and transcription opens the locus in photoreceptor precursors. Of these precursors, 67% express *ss* at high levels and 33% express at low levels. After transcription ceases, chromatin compacts in a subset of cells which eventually give rise to Ss^{OFF} R7s. In the cells that remain open, *ss* expression is re-activated by the *late enhancer* and these cells become Ss^{ON} R7s. We hypothesize that differential *spineless* expression in precursors drives the terminal Ss^{ON}/Ss^{OFF} R7 fate choice: high *ss*-expressing precursors become Ss^{ON} R7s and low *ss*-expressing precursors differentiate into Ss^{OFF} R7s. We developed reporters including the MS2 MCP system to monitor *ss* transcription initiation, elongation, and termination and the ParB/*parS* and LacI/*lacO* systems to track live chromatin compaction. Using these tools, we will investigate how variability in transcription and chromatin is harnessed to diversify cell types.

1342F **Coordinating stereotyped and stochastic patterns in the *Drosophila* eye** Alison Ordway[1], Caitlin Anderson[2], Lukas Voortman[2], Elizabeth Urban[1], Robert J Johnston[1] [1]Biology, Johns Hopkins University, [2]Johns Hopkins University

During development, stereotyped patterning produces nearly identical structures across individuals of the same genotype. In contrast, stochastic cell fate specification produces random patterns that are unique to each individual. How gene regulatory mechanisms are coordinated to generate highly regular patterns and stochastic patterns within the same tissue is poorly understood. Here, we address this question in the context of the developing *Drosophila* eye. The fly eye has a stereotypical array of photoreceptors that arises through a wave of morphogenesis driven by Hedgehog (Hh) signaling. Overlaid on this highly uniform structure is a random pattern of two R7 photoreceptor subtypes, controlled by stochastic ON/OFF expression of the transcription factor, Spineless (Ss). Here, we find that Hh regulates *ss* during stochastic R7 subtype patterning. *hh* mutants display reduction in the size of the eye and the proportion of Ss^{ON} R7s. Excitingly, Cubitus Interruptus (Ci), an effector of Hh signaling, does not seem to play a major role in regulating *ss* expression, suggesting a non-canonical mechanism of action for Hh signaling. We are investigating targets of the Hh signaling pathway including Decapentaplegic (Dpp) and Homothorax (Hth), as well as factors involved in cell cycle regulation, to identify mechanisms that regulate *ss* expression downstream of Hh signaling. Our data suggest that Hh signals from differentiating photoreceptors to activate *ss* expression in immature precursor cells, similar to how Hh signals to promote morphogenesis of the fly eye. Thus, Hh signaling coordinates the generation of stereotyped eye morphology and stochastic R7 subtype patterning during development.

1343F **BRWD3 promotes KDM5 degradation to maintain H3K4 methylation levels** Dongsheng Han[1], Samantha H Schaffner[1], Jonathan P. Davies[1], Mary Lauren Benton[2], Lars Plate[1,3], Jared Nordman[1] [1]Department of Biological Sciences, Vanderbilt Univeristy, [2]Department of Computer Science, Baylor University, [3]Department of Chemistry, Vanderbilt Univeristy

Histone modifications are essential for regulating chromatin dynamics and gene expression. Disruptions in histone modifications levels likely contribute to diseases like cancer. H3K4 methylation is a known histone modification that associated with active gene transcription. While enzymes that add or remove methyl groups from H3K4 are known, how cells balance these enzyme levels to maintain normal H3K4 methylation levels is unclear. Here, we show that the chromatin-binding protein BRWD3 (Bromodomain and WD repeat-containing protein 3), a substrate-specificity factor of the Cul4-DDB1 E3 ubiquitin

ligase complex, helps balance H3K4 methylation levels through ubiquitin-proteasome system-dependent proteolysis. We found that depleting BRWD3 not only causes an increase in H3K4me1 levels but also causes a decrease in H3K4me3 (H3 lysine 4 trimethylation) levels, indicating that BRWD3 broadly influences H3K4 methylation. Using immunoprecipitation coupled to quantitative mass spectrometry, we identified an interaction between BRWD3 and the H3K4-specific lysine demethylase 5 (KDM5/Lid), an enzyme that removes tri- and dimethyl marks from H3K4. Moreover, analysis of ChIP-seq (chromatin immunoprecipitation sequencing) data revealed that BRWD3 and KDM5 are significantly colocalized throughout the genome and H3K4me3 are highly enriched at BRWD3 binding sites. We show that BRWD3 promotes K48-linked polyubiquitination and degradation of KDM5 and that KDM5 degradation is dependent on both BRWD3 and Cul4. Critically, co-depleting KDM5 restores altered H3K4me3 levels upon BRWD3 depletion. We also show that BRWD3 is a PEV (position-effect variegation) gene and genetically interacts with KDM5. Together, our results demonstrate that BRWD3 promotes KDM5 degradation to balance H3K4 methylation levels. Future works will focus on how BRWD3 regulates KDM5 activity throughout development and its broader implications for epigenetic programming and cell differentiation processes.

1345F **It's *abo*ut time: an investigation into the role of *abnormal oocyte* (*abo*) localization, function, and interaction in embryonic histone gene regulation** Eric H Albanese, Casey Schmidt, Leila E Rieder Biology, Emory University

Abnormal oocyte (abo) is a maternal-effect gene first characterized by Larry Sandler in 1970. Loss-of-function mutations in *abo* cause defects in embryonic development. Subsequent characterizations of abo revealed that it negatively regulates histone gene expression, likely through the targeting of *histone* promoters. Interestingly, the viability defects resulting from abo mutations can be rescued by overexpression of heterochromatic regions, suggesting that heterochromatin can act as a «sponge» to absorb excess histones. Despite these important experiments, *abo* has received little attention since the early 2000s, and its precise role in histone repression remains unknown. Therefore, our goal is to define the molecular role of abo in histone gene expression and promote a clearer understanding of negative histone regulation in *Drosophila melanogaster*. To achieve this, we generated V5-tagged *abo* lines via CRISPR. Using these lines, we analyzed abo localization in polytene chromosomes and embryos. We found that Abo does not behave as previously characterized in polytene chromosomes at endogenous or over-expressed levels. However, we find, novelly, that syncytial embryos do show Abo localizes to the histone genes. Our next experiments will focus on modulating *abo* expression via genetic drivers, null mutants, and transgenic histone-array rescue lines to determine the effect on histone expression at the mRNA and protein level. We will also compare Abo to Mute, another negative regulator of histone expression to elucidate if Abo and Mute have similar mechanisms of action, and if they can functionally compensate for each other. Lastly, we seek to broaden our analysis beyond historically relevant tissues (such as salivary glands), as we seek to propose a novel candidate for Abo activity, the early embryo. This research will contribute to an understanding of negative regulation of histone genes.

1346F **Regulation of Spermatogenesis by the BTB Transcription Factor Ribbon** Madeline Hakala, Christine Severude, Jennifer Jemc Loyola University Chicago

Past research has demonstrated that the transcription factor Ribbon (Rib) plays an essential role in *Drosophila* testis development and function. Loss of function of *rib* results in a failure of somatic cells to coalesce to form a gonad in the developing embryo. More recent work from our lab has shown that increased expression of *rib* throughout development results in significant morphology defects in the adult testis. If overexpression is limited to germ cells and somatic cells in the adult, more mild defects are observed. Ultimately, we are interested in identifying Rib transcriptional targets in this context to understand how it regulates spermatogenesis. To achieve this, we began by conducting RNA-sequencing of both control testes and testes in which *rib* was overexpressed in somatic cells. Utilizing a standard differential expression analysis pipeline, gene expression levels of *rib* overexpression flies were compared to the control, resulting in the identification of approximately 300 genes with statistically significant changes in gene expression after results were filtered. Given the previously described role of Rib in the regulation of cell morphogenesis and cell-cell interactions during development, we were particularly interested in genes involved in cell adhesion and regulation of the cytoskeleton. At this time, we have confirmed that *shortstop* (*shot*), which encodes a cytoskeletal linker protein, is a transcriptional target of Rib, revealing that Rib may regulate spermatogenesis through control of the cytoskeleton. Additional validation experiments are currently being conducted for other genes encoding cell adhesion proteins and regulators of the cytoskeleton to further support the role of Rib in regulating cell shape changes throughout spermatogenesis.

1347F **Exploring the function of Kelch stop codon readthrough protein in the Drosophila brain** Yaqing Cheng[1], Andrew Hudson[2], Lynn Cooley[2] [1]Genetics, Yale University, [2]Genetics, Yale university

Stop codon recognition is essential for proper protein synthesis termination, but under specific conditions, stop codons are recognized as sense codons, enabling continued translation. This process, known as stop codon readthrough (SCR), is conserved across various organisms, including viruses, yeast, Drosophila, and mammals. While more than 400 genes in Drosophila have been predicted to undergo SCR, only a limited number have been experimentally validated and characterized.

We have discovered that SCR is subject to tissue and cell type-specific regulation, with the highest efficiency observed in the Drosophila brain. Our previous research identified Kelch, a substrate adaptor for the Cullin Ring E3 ubiquitin ligase, as a gene undergoing SCR in Drosophila, producing an evolutionarily conserved C-terminal extension. To elucidate the functional significance of Kelch readthrough, we used newly engineered promiscuous ligases, TurboID coupled with mass spectrometry to identify interacting molecules associated with Kelch readthrough protein (KelRT) in the brain. Our analysis has revealed 64 high-confidence interacting partners of KelRT, some of which play roles in nervous system development and response to external stimulus. Additionally, we utilized CRISPR-Cas9 HDR to introduce the QF protein at the C-terminus of Kel-ORF2, enabling us to map Kelch SCR-expressing neuronal cell types through in combination with neuronal-specific Gal4 drivers. In summary, our research aims to uncover the functional role of KelRT in Drosophila brains, contributing to a better understanding of the significance of SCR.

1348F **Transcriptomic Analysis of Phenotypic Non-Specificity in *Drosophila melanogaster*** Gabriella Sidhu, Anthony Percival-Smith Biology, University of Western Ontario

Phenotypic nonspecificity of transcription factor (TF) function is the redundant rescue of a TF phenotype by multiple non-resident TFs that recognize distinct DNA-binding sites. In *Drosophila melanogaster*, phenotypic non-specificity is frequently observed across many TF phenotypes of which two are important for this study. The Apterous (AP) transcription factor is expressed in the cells of the wing imaginal disc dorsal compartment and is required for determination of the dorsal compartment and adult wing development. The *ap* null wingless mutant phenotype is rescued by expression of both the resident TF AP and by three non-resident TFs, Caudal (CAD), Tramtrack (TTK) and Myb oncogene-like (MYB). The Doublesex female (DSX^F) TF is expressed in abdominal cells and is required for female differentiation. Female pigmentation, characterized by an abdominal zebra stripe-like pattern of pigmentation, can be rescued in *dsx* null mutants by the expression of DSX^F and four non-resident TFs: Antennapedia (ANTP), Eyeless (EY), Bric à brac 1 (BAB1), and Odd-skipped (ODD), but not by expression of Forkhead box O (FOXO), and Squeeze (SQZ). ANTP, EY and ODD are not required for normal female abdominal pigmentation. DSX^F activates BAB1 expression, and ANTP, EY, ODD, have been shown to either substitute for, or work downstream of, BAB1. I am using the AP and DSX^F examples of phenotypic non-specificity to determine the sets of genes regulated by resident and non-resident TFs. The sets of regulated genes are identified using RNAseq, and compared to distinguish between three possible patterns: independence, partial overlap, or co-regulation. DSX RNAseq data has shown that the patterns of genes regulated by DSX^F, BAB1, ANTP, ODD and EY partially overlap with one another, and indeed the genes regulated by SQZ and FOXO which do not rescue abdominal pigmentation also partially overlap with all other TFs tested. RNAseq and subsequent characterization of the regulated sets of genes of the AP resident and non-resident TFs is currently in progress. The results of both examples of phenotypic non-specificity will, if similar, limit the models that can be used to explain phenotypic nonspecificity and the regulation of the initiation of transcription.

1349F **RNA-binding Protein Nocte Regulates mRNA translation and decay during *Drosophila* Development** Tianyi Zhang[1], Yutong Xue[1], Jennifer Martindale[1], Seung-Kyu Lee[2], Weiping Shen[3], Myriam Gorospe[3], Weidong Wang[3] [1]Laboratory of Genetics and Genomics, National Institute on Aging, [2]Laboratory of Genetics and Genomics, National Institute of Aging, [3]National Institute of Aging

RNA-binding proteins (RBPs) play essential roles in mRNA splicing, translation, and decay. To study the functions of an RBP and identify its targets, it is important to have reliable methods to detect the genome-wide changes at mRNA levels and at translation levels. Furthermore, the methods need to be sensitive enough for the samples that are laborious and difficult to collect, such as specific fly tissues. RNA-seq is an established method to measure mRNA levels with small amounts of samples, while Ribo-seq (also known as ribosome profiling) can detect ribosome footprints of the mRNAs to measure their translation efficiencies (TEs). In our recent studies of the RBP Nocte, we adapted the current Ribo-seq method and quantitatively detected ribosome footprints in *Drosophila* eye discs. Knockout of *nocte* leads to lethality, and its eye-specific depletion impairs eye size and morphology. Our Ribo-seq and RNA-seq results in eye discs show that Nocte preferentially enhances translation of mRNAs with long upstream open reading frames (uORFs). One of the key Nocte targets, *glass* mRNA, encodes a transcription factor critical for differentiation of photoreceptor neurons and accessory cells. Mechanistically, Nocte counteracts long uORF-mediated translational suppression of *glass* by promoting translation reinitiation downstream of the uORF. We are currently studying the mechanisms of mRNA decay caused by translation suppression in Nocte-depletion eye discs and the functions of Nocte in brains, forguts/midguts, hindguts/malpighian tubules and fat bodies with RNA-seq and Ribo-seq methods.

1350F **Lysine 36 of *Drosophila* histone H3.3 supports adult longevity** Benjamin D. McMichael[1,2], John C. Brown[2], Vasudha Vandadi[2], Aadit Mukherjee[1], Harmony R. Salzler[2], A. Gregory Matera[1,2,3,4] [1]Department of Biology, University of North Carolina at Chapel Hill, [2]Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, [3]Department of Genetics, University of North Carolina at Chapel Hill, [4]RNA Discovery Center, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

Aging is a multifactorial process that disturbs homeostasis, increases disease susceptibility, and ultimately results in death. Although the definitive set of molecular mechanisms responsible for aging remain to be discovered, epigenetic change over time is proving to be a promising piece of the puzzle. Several posttranslational histone modifications (PTMs) have been linked to the maintenance of longevity. Here, we focus on lysine 36 of the replication-independent histone protein, H3.3 (H3.3K36). To interrogate the role of this residue in *Drosophila* developmental gene regulation, we generated a lysine to arginine mutant that blocks the activity of its cognate modifying enzymes. We found that an *H3.3B*^{K36R} mutation causes a significant reduction in adult lifespan, accompanied by dysregulation of the genomic and transcriptomic architecture. Transgenic co-expression of wild-type *H3.3B* completely rescues the longevity defect. Because H3.3 is known to accumulate in non-dividing tissues, we carried out transcriptome profiling of young vs aged adult fly heads. The data show that loss of H3.3K36 results in age-dependent misexpression of NF-κB and other innate immune target genes, as well as defects in silencing of heterochromatin. We propose H3.3K36 maintains the postmitotic epigenomic landscape, supporting longevity by regulating both pericentric and telomeric retrotransposition and by suppressing aberrant immune signaling.

1351F **The requirement of Dosage Compensation in Drosophila development and aging** Kaitlyn M Cortez, Gunjan Singh, Nathan Ningrin, Zining Chen, Erica Larschan Molecular Biology, Cell Biology, and Biochemistry, Brown University

Dosage compensation (DC) is an essential conserved process that amplifies the expression of thousands of genes encoded on the single male X-chromosome two-fold. Although the onset of Dosage Compensation is at the mid-embryo stage, it is not known when DC is required for proper development and aging because it has not been knocked down at specific time points. Thus, I will knock down DC function in Drosophila embryos, larvae, and adults by disrupting the MLE component (RNA helicase A) of the master regulator of DC, the Dosage Compensation Complex (DCC). Utilizing the MLE-CRY2 optogenetic system to knock down MLE will allow me to investigate the effects of DC disruption at different time points while bypassing the early lethality seen in males. I will analyze sex-specific survival and longevity to investigate the role of DC in development and aging. My preliminary data suggests that DC is continuously required for the development of embryos into larvae and normal lifespan in adult males.

1352F **Ectopic heterochromatin triggered by insertion of repetitive DNA is temperature-sensitive** Melissa K. Sawyer, Alix Hathaway, Andrew M. Arsham Bemidji State University

Heterochromatin is a key genomic defense against invasive genetic elements, mitigating damage by inhibiting transposition, silencing gene expression, and reducing recombination at insertion sites. How a cell's epigenetic machinery recognizes and neutralizes novel threats, prior to establishing sequence-specific adaptive defenses like piRNAs is poorly understood. Here we show that the recognition and silencing of an exogenous tandem array of repetitive DNA is highly location-dependent. To investigate genome defense against novel repetitive DNA we carried out a transposition mutagenesis screen, mobilizing a reporter construct expressing the white gene adjacent to a 256-copy tandem array of the lac operator sequence from E. coli. We isolated insertional mutants with variegated eye color suggesting silencing by heterochromatin. Most recovered transgenic flies had full reporter gene expression and uniform red eye color - only ~1% of recovered flies had variegated eye color, indicating that the repeat array was not itself sufficient to trigger silencing. Surprisingly, the observed variegation is exceptionally sensitive to rearing temperature despite different genomic locations and chromatin contexts. Suppression of variegation at lower growth temperatures may provide clues to the genetic and biochemical mechanisms of genome defenses against novel invasive DNA. A clearer understanding of what cis-acting genomic features distinguish euchromatin that is sensitive to repeat-induced silencing from that which is not will provide insight into genomes' innate pathogen-sensing mechanisms as well as senescence and disease caused by disordered heterochromatin.

1353F **Determining the Regulatory Effects of Wolbachia on Heterochromatin in Drosophila Melanogaster** Nkechi Aman, Andrew Arsham Bemidji State University

Wolbachia is an intracellular bacterial parasite common in insects including Drosophila and mosquitoes. Wolbachia infection has profound effects on the metabolism, gene expression, and reproduction of insects and is poised to become an important tool to combat mosquito-borne diseases. Because Drosophila melanogaster is a common host for Wolbachia, the tools of Drosophila genetics can be used to understand the host- parasite relationship. Wolbachia has recently been shown to increase homologous recombination in D. melanogaster, but the mechanism by which it accomplishes this is unknown. We hypothesized that because heterochromatin inhibits recombination, it could be playing an intermediary role in Wolbachia's effects on recombination. Female flies from two wild type lines from the Drosophila Genetic Reference Panel that normally carry Wolbachia, and genetically matched controls that have been cured of Wolbachia by antibiotic treatment, were crossed with PEV flies and the ability of Wolbachia infection to modify the PEV phenotype was assessed. We find that, while there is marked variation between the two DGRP lines and between the different PEV stocks, Wolbachia infection can suppress variegation, suggesting that Wolbachia can inhibit heterochromatin formation pointing towards a possible mechanism for

Wolbachia's effect on recombination.

1354F **Brain specific microRNA mediated regulation of fat storage via Neuropeptide like Precursor 1 in *Drosophila melanogaster*** Pushpa Sharma, Davie Van Vactor Cell Biology, Harvard Medical School

Metabolism is a complex process. To maintain an optimal metabolic balance, an organism needs to match its food intake and energy storage in the form of fat with appropriate energy mobilization. These metabolic processes need to be finely regulated to fulfill timely physiological needs and to maintain the overall well-being of an organism. Misregulation of metabolism often results in diseases such as obesity, diabetes, cardiovascular diseases, or elevated triglycerides and cholesterol, which cause high mortality throughout the current world population. Small non-coding microRNAs (miRNAs) are well-suited as regulators of physiological and adaptive biological processes that require rapid and/or local control over gene expression. miRNAs control gene expression by inhibiting protein translation and destabilizing mRNA targets.

In our current research, we have been exploring the brain specific microRNA regulation of metabolic processes using *Drosophila melanogaster* as a model system. We have recently identified a novel neural circuit that controls metabolic homeostasis in *Drosophila* by a conserved Central Nervous system (CNS)-specific miRNA, *miR-1000* (ortholog of human *miR-137*). We find that miR-1000 regulates body weight, fat storage, survival under nutrient deprivation, and longevity, by targeting the Neuropeptide Like Peptide 1 (*Nplp1*) gene in the CNS. Loss of miR-1000 and consequent increase in Nplp1 expression results in flies with increased fat storage and extended survival in the absence of nutrients. The biological function of Nplp1 neuropeptides is largely unknown. Here, we define an important and novel biological function of *Nplp1* in maintaining metabolic homeostasis in *Drosophila*. miR-1000 and Nplp1 levels also change dynamically during refeeding after a period of starvation, suggesting an important adaptive function in metabolism.

1355F **Antisense transcription within the *histone* locus feeds into small RNA pathways and contributes to regulating *histone* mRNA expression during embryogenesis** Samantha A Russell[1,2], Fabio A Lefebvre[1,3], Ashley Chin[1,4], Louis PB Bouvrette[1,3], Julie Cardin[1], Gabrielle Deschamps-Francoeur[1], Eric Lécuyer[1,3,4] [1]RNA biology, IRCM, [2]Experimental Medicine, McGill, [3]Université de Montréal, [4]McGill

Endogenous small interfering RNAs (endo-siRNAs) are generated from a variety of sources, including long structured loci, transposable elements, and natural antisense transcripts (NATs). NATs are expressed from many loci in several organisms, including from a few of the *histone* (*his*) loci in *Drosophila, C. elegans,* and *Leishmania major*. In *Drosophila, his* mRNAs are encoded by tandemly arrayed gene units of the five *his* genes, and we find NATs express from all five *his* loci. Similar to *his* mRNAs, *his* NATs lack a polyA tail and transcriptomic analysis of mutant embryos indicates that dSLBP is necessary for *his* NAT expression. To characterize where *his* NATs localize to within the cells in the early embryo, we utilized a subcellular fractionation approach followed by RNA deep-sequencing. We find that *his* NATs are more cytoplasmic and mimic their mRNA counterparts, which suggests that *his* NATs contribute to the formation of double stranded precursors leading to siRNA molecules. Consistent with this idea, siRNA precursors are cleaved to 21-nt lengths during biogenesis, and small RNA-sequencing found small RNA reads at all *his* genes, with peaks at 21-nt lengths. In addition, analysis of RNA-seq data of small RNAs pulled down with AGO2, but not with AGO1, shows an enrichment of reads derived from the *his* locus. Finally, RNA-seq on embryo progeny from *AGO2^{414}/Df* mutant females reveals that loss of AGO2 specifically upregulates several *his* mRNAs, which suggests that AGO2-linked *his* endo-siRNAs contribute to the clearance of maternal *his* mRNAs during early *Drosophila* embryogenesis. Altogether, this work identifies a group of NATs, describes how they are regulated, characterizes where they localize to within the cell, and provides evidence that *his* NATs generate *his* endo-siRNA. We propose a working model where *his* NATs generate siRNA that interact with AGO2 in the early embryo, and target their corresponding genes to clear maternal *his* mRNAs.

1356F **Murine genetic reference population reveals the regulation and function of *Rpl3l* in the heart** Akhilesh K Bajpa[1], Qingqing Gu[1], Buyan-Ochir Orgil[2,3], Neely Alberson[2], Jeffrey A Towbin[2,3], Enkhsaikhan Purevjav[2,3], Lu Lu[1] [1]Genetics, Genomics and Informatics, University of Tennessee Health Science Center, [2]Pediatrics, University of Tennessee Health Science Center, [3]Children's Foundation Research Institute, Le Bonheur Children's Hospital

Background: Cardiomyopathy, a disease of heart muscles, is a common cause of heart failure. Recently, biallelic variants in ribosomal protein L3-like (*RPL3L*) have been reported in patients with dilated cardiomyopathy (DCM) and heart failure. This study aimed to employ a systems genetics approach to understand the regulatory mechanisms underlying *RPL3L* function in DCM.

Methods: The well-known murine genetic reference population, the BXD mice were used for genetic correlation and expression quantitative trait loci (eQTL) mapping. DCM patient and normal heart samples were used for identifying

differentially expressed genes. Functional enrichment analyses of the *Rpl3l*-correlated genes in BXD heart and differential genes in DCM patient versus normal heart samples were performed. Cardiac gene expression and echocardiography parameters in BXD mice were correlated to understand gene-phenotype associations. Furthermore, infiltration analysis of 24 different immune cells was performed using DCM patient and control heart gene expression data to reveal the associations between DCM, immune cell types and *RPL3L* expression.

Results: Systems genetics analysis identified high expression of *Rpl3l* mRNA, which ranged from 11.31 to 12.16 across BXD mice with ~1.8-fold difference. Pathways such as "DCM", "focal adhesion", "MAPK signaling" and "PI3K-Akt signaling" were significantly associated with *Rpl3l*-correlated as well as differentially expressed genes. The eQTL mapping suggested *Myl4* (Chr 11) and *Sdha* (Chr 13) as the upstream regulators of *Rpl3l*. The mRNA expression of *Rpl3l, Myl4* and *Sdha* was found to be significantly correlated with different echocardiography traits, such as ejection fraction, fractional shortening, cardiac output, left ventricular volume and end-systolic diameter in BXD mice. Immune cell infiltration analysis revealed a significant association of *RPL3L* and *SDHA* with seven immune cells (CD4 and CD8 naïve T, CD8 T, macrophages, cytotoxic T cell, Gamma delta T cell and Exhausted T cell) that were also differentially infiltrated between DCM patient and normal heart samples.

Conclusions: Expression of *Rpl3l* and its upstream regulators, *Myl4* and *Sdha,* correlate with multiple cardiac function traits in BXD mice, while *RPL3L* and *SDHA* correlate with immune cell infiltration in DCM patient hearts. These results suggest important roles for *RPL3L* in the development of DCM and heart failure.

1357F **Increased hemimethylation levels correlate with methylation reductions in DNA methyltransferase mutant mouse embryos** Chloe Tang[1], Tamara Davis[2] [1]Bryn Mawr College, [2]Biology, Bryn Mawr College

Mammals have evolved to have many forms of gene regulation. Genomic imprinting is one form that only allows the expression of one parental allele from an organism's genome. Imprinted genes are regulated epigenetically through DNA methylation, facilitated by DNA methyltransferase (DNMT1), where a methyl group (CH_3) is added to one parental allele to generate a differentially methylated region (DMR). Differential methylation results in differential gene expression of the parental alleles and the proper establishment of methylation patterns is crucial for embryonic development as abnormal methylation patterns can lead to inappropriate gene expression, developmental disorders, and diseases such as cancer. Methylation at primary DMRs, which is inherited at fertilization, is stable and symmetric throughout development whereas methylation at secondary DMRs, which is acquired during early embryogenesis, is less stable and asymmetric. In addition, previous research suggested that methylation is well maintained at primary DMRs in mouse embryos with a hypomorphic mutation of *Dnmt1* but is dramatically reduced at secondary DMRs and non-imprinted loci. These pieces of evidence suggest that DNMT1 might function differently at different loci. We hypothesize that reduced DNMT1 fidelity leads to an increase in hemimethylation, resulting to the observed methylation loss at secondary DMRs and non-imprinted loci. To test this hypothesis, I am analyzing the methylation patterns at both non-imprinted and imprinted loci in 12.5 days post conception (dpc) wild-type and *Dnmt1* mutant mouse embryos. Analysis of methylation patterns at three paternally methylated loci associated with imprinted genes indicated that the paternally methylated primary DMR was minimally affected but methylation was dramatically reduced at both secondary DMRs in *Dnmt1* mutant vs. wild-type embryos. In addition, the hemimethylation level at secondary DMRs was significantly higher in mutants than in wild type but this difference was less pronounced at the primary DMR. This inverse correlation between methylation and hemimethylation levels suggests that DNMT1 is essential in maintaining methylation patterns, especially regarding methylation symmetry during fetal development and that its inconsistent function leads to a loss of methylation and increase in hemimethylation. I'm currently extending my analysis to maternally methylated DMRs and non-imprinted loci to see if the pattern is generalizable.

1358F **Evolution of programmed DNA elimination in parasitic nematodes** James R Simmons[1], Brandon Estrem[1], Maxim V Zagoskin[1], Ryan Oldridge[1], Sobhan Zadegan[2], Jianbin Wang[1,2] [1]Biochemistry & Cellular and Molecular Biology, The University of Tennessee, [2]Genome Science & Technology, The University of Tennessee

A growing list of metazoans undergo programmed DNA elimination (PDE), where a significant amount of DNA is selectively lost from the genome during development. In nematodes, PDE leads to the removal of the ends of all germline chromosomes. In some species, PDE also eliminates sequences in the interior of the chromosomes, leading to an increased number of somatic chromosomes. The biological significance of these karyotype changes associated with PDE and the origin and evolution of nematode PDE remain largely unknown. Here, we assembled the single pair of germline chromosomes of the horse parasite *Parascaris univalens* and compared the karyotypes, gene organization within the chromosomes, and PDE features among ascarids. We showed that *Parascaris* converts an XX/XY sex-determination system in the germline into an XX/XO system in the somatic cells. Our analyses of the parasitic nematodes *Ascaris*, *Parascaris,* and *Baylisascaris* suggest that PDE existed in the ancestor of these parasites, and their current drastically different germline karyotypes were derived from fusion events of the same ancestral smaller chromosomes. PDE resolves these fused germline chromosomes and restores their pre-fusion

karyotypes, leading to alterations in genome architecture and gene expression in the somatic cells. Cytological and genomic analyses further reveal the dynamic organization of the *Parascaris* germline chromosome during meiosis and a potential function for the satellite DNA and the heterochromatin arms. Overall, our results show that PDE enables two karyotypes to exist within an individual, with both the fused germline chromosomes and the split somatic chromosomes contributing to genome organization and functions.

1359F **Sulforaphane disrupts lepidopteran epigenetic systems** Marisa Arreola, Ashley Groff, Whitney Finney, David B. Kushner, Thomas M. Arnold, Dana J. Somers Biology, Dickinson College

Sulforaphane (SFN), an inhibitor of histone deacetylases (HDACs), has been seen to disrupt enzyme activity, DNA-histone binding, and gene expression upon consumption in humans. In an ecological context, SFN is produced by cruciferous plants and works as a defense against grazers by disrupting their epigenetic systems. Experiments in different insect species showed that SFN reduces HDAC activity and slows the development of the beet armyworm (*Spodoptera exigua*), a generalist feeder. In contrast, the cabbage looper (*Trichoplusia ni*), a specialist that feeds exclusively on SFN-producing plants, has partial resistance to the effects of SFN. Experiments demonstrated that SFN slowed HDAC activity and growth of *S. exigua* whereas there was no significant change in *T. ni.* The gene expression of both grazers was also examined using RNA-seq analysis to classify genes and map them to metabolic pathways. Genes involved in energy production were downregulated in *S. exigua* that fed on SFN. To test whether SFN had transgenerational effects, a second generation of *S. exigua* and *T. ni* were produced from the generations that fed on SFN. Initial data suggests that SFN had minimal transgenerational effects in both species. This research suggests that SFN, and HDAC inhibitors in general, disrupt epigenetic systems within grazers who consume cruciferous plants, which could have broader implications for human health.

1360F **Revealing early cell cycle dynamics in epithelial ovarian cancer cells with CDK4/6 inhibition** Karl L Schneider[1], Paul D Goetsch[2] [1]Biology, Michigan Technological University, [2]Michigan Technological University

Despite decades of research and the development of initially effective treatment strategies, epithelial ovarian cancer remains a leading cause of cancer death for women worldwide. Quiescent, stem-like side populations of cancer cells within the tumor microenvironment could be the culprit behind the recurrence of the disease following remission in the majority of ovarian cancer patients . The pocket proteins pRb and p130, along with the DREAM complex, coordinate the repression of late G1 and S phase gene expression which is essential for maintaining quiescence. Cell cycle regulatory proteins are often mutated during oncogenesis, and it remains unclear what minimal functional components are necessary for cancer cells to retain the ability to quiesce. We used the CDK4/6 inhibitors palbociclib, abemaciclib, and ribociclib to induce quiescence and synchronize populations of cell lines in culture (2 epithelial ovarian cancer cell lines SKOV3 and HEYA8 and 1 immortalized primary fallopian tube epithelial cell line FT282). We performed flow cytometry and western blotting to confirm the effective induction of quiescence following treatment with CDK4/6 inhibitors. Analysis of the transcriptome via mRNA sequencing revealed the genes actively expressed as well as the relative changes in gene expression during and following this period of artificially induced quiescence. Finally, chromatin immunoprecipitation exposes the spatiotemporal dynamics of DNA binding by relevant transcription factors, including pRb and the DNA binding components of the DREAM complex. Taken altogether, these analyses enable a deeper understanding of the mechanisms underlying quiescence in epithelial ovarian cancer. Also, the establishment of a model for cell cycle synchronization with CDK4/6 inhibition and the subsequent analytical pipelines described herein can be expanded to later phases of the cell cycle, protein targets within other relevant signaling pathways, and other types of cancer. Novel data pertaining to the maintenance of quiescence in epithelial ovarian cancer will hopefully contribute to the development of better diagnostics and treatments for the detection and prevention of recurrent tumors.

1361F **Small RNAs in Nematode Programmed DNA Elimination** Maxim Zagoskin[1], Richard E. Davis[2], Jianbin Wang[3] [1]BCMB, The University of Tennessee, Knoxville, [2]Biochemistry and Molecular Genetics, University of Colorado School of Medicine, [3]Biochemistry & Cellular and Molecular Biology, The University of Tennessee, Knoxville

Programmed DNA elimination (PDE) is an exception to the DNA constancy rule, where an individual's germline and somatic genomes maintain identical DNA content. In some metazoans, nuclear DNA is selectively excised from the somatic genome, leaving the germline genome intact. In the parasitic nematode *Ascaris*, PDE leads to comprehensive chromosome end-remodeling, increased chromosome number, and loss of 18% of the germline genome including ~1000 genes. The mechanisms governing PDE and its broader implications for genome stability and metazoan evolution remain largely enigmatic.

Small RNAs are known to play a role in ciliate PDE. However, PDE is diverse and has distinct features in different taxa. Here we examined small RNAs in a nematode undergoing PDE, to ascertain their association with retained, eliminated DNA, or DNA breaks. We identified Argonaute proteins in *Ascaris* and characterized small RNAs associated with seven of these Argonautes. Immunostaining revealed that two of these Argonaute proteins, WAGO-2 & WAGO-3, are associated with PDE. Notably,

WAGO-3 staining was found to be enriched on eliminated DNA which was corroborated by ChIP-seq data. Through RNA chromatin immunoprecipitation assay, we identified specific sets of small RNAs that associate with nascent RNAs specific to WAGO-3 in the eliminated regions. These WAGO-3 secondary small RNAs are likely generated from primary siRNAs originating from double-stranded RNA derived from extensive bidirectional transcription in eliminated regions.

We suggest WAGO-3-associated small RNAs mark eliminated regions, binding to nascent RNAs on eliminated chromatin, thereby regulating gene expression in these regions. Conversely, WAGO-2 predominantly stains retained regions during the PDE process, and a subset of its associated small RNAs specifically targets repetitive sequences within these retained regions. WAGO-2 small RNAs may directly interact with the repetitive chromatin within the retained regions to ensure the suppression of potentially deleterious repeats during PDE. In conclusion, our data suggests that two Argonaute proteins, WAGO-2 and WAGO-3, along with their associated small RNAs, distinguish retained and eliminated DNA by selectively targeting specific chromosome regions, possibly in response to chromatin changes triggered by PDE.

1362F **Function and regulation of the Mediator complex kinase subunit Cdk8** Sara McPherson[1], Bhuvan Anbalagan[1], Mary-Elizabeth Raymond[1], Ivan Sadowski[2], Maria Aristizabal[3] [1]Biology, Queen's University, [2]University of British Columbia, [3]Queen's University

In eukaryotes, transcription is performed by one of several RNA polymerases (RNAP), RNAPII synthesizing the most significant portion of RNAs. RNAPII transcription regulation is complex, as such, is poorly understood. The Mediator complex is a significant transcriptional regulator, organized into the head, middle, tail, and the dissociable Cdk8 kinase module. Cdk8 is a Mediator subunit and an evolutionarily conserved kinase with well-known roles in transcriptional activation and repression. These transcriptional activities are mediated by Cdk8's ability to phosphorylate several components of the transcriptional machinery and sequence-specific transcription factors. In budding yeast, this includes the RNAPII C-terminal domain (CTD), several Mediator complex subunits, and the sequence-specific transcription factors Gal4, Gcn4, Ste12, and Phd1. For example, Cdk8 can repress context-specific gene expression by phosphorylating Gcn4, Ste12, and Phd1, targeting them for proteolytic degradation and blocking the activation of their respective target loci in response to nutrient availability. By contrast, Cdk8 activates transcription by phosphorylating Gal4 and its target loci in response to the presence of galactose in the growth media. Although well studied, deletion of Cdk8 in budding yeast produces more gene expression alterations than can be accounted for by its known substrates. Findings suggest an incomplete understanding of the factors that Cdk8 targets to regulate transcription, and how Cdk8 activity is regulated remains unknown. My project leverages recent structural insights and results from our recent high-resolution gene expression time course analysis before and several time points after nuclear depletion of Cdk8 to close these knowledge gaps. This work will reveal significant aspects of Cdk8 function, uncovering mechanisms by which organisms respond to changing environments. Understanding how Cdk8 regulates transcription will also show how a single factor can integrate several distinct signals to produce tightly regulated transcriptional outputs, a fundamental question in the field. Revealing aspects of normal Cdk8 function will also shed insight into the role of Cdk8 as an oncogene and tumor suppressor, informing efforts to develop Cdk8 inhibitors for anti-cancer treatment.

1363F **The exonic promoter of *Saccharomyces cerevisiae HKR1*, which encodes a mucin-like multidomain protein, makes for divers expression patterns** Toshihiro Kondo, Ryotaro Ozawa, Yuna Hosokawa, Shin Kasahara Food & Agricultural Sciences, Miyagi University

HKR1 was originally isolated from the genome of *Saccharomyces cerevisiae* as a gene that confers resistance to HM-1 killer toxin produced by the killer yeast *Lindnera mrakii* (synonym *Hansenula mrakii*). *HKR1* is an intronless gene with a 5.4 kb ORF encoding a mucin-like multidomain transmembrane protein, Hkr1p. Hkr1p contains a consensus sequence of EF hand, a calcium-binding motif and the DNA-binding leucine zipper motif in its cytoplasmic tail, and has actually been known as an osmosensor of the HOG MAP kinase complex.

We recently found that *HKR1* has another cryptic promoter in its exon and is transcribed not only from the promoter in the 5' upstream region but also from the region around the 3330th nucleotide (nt. #3330) from the translation initiation site. In addition, it has been confirmed that the transcriptional activity of the exonic promoter is silenced by its upstream sequence within the exon of *HKR1*. In this study, we investigated whether the suppressed transcription is restored by external conditions such as osmotic pressure by using reporter assay systems.

Plasmids containing various length of the exonic promoter region were constructed and a fluorescence protein gene or the *lacZ* gene of *Escherichia coli* was ligated to each promoter sequence, introduced into *S. cerevisiae* cells, then expression levels were evaluated by measuring the fluorescence intensity or beta-galactosidase activity. The maximum transcriptional activity was observed when the reporter genes were ligated to the 360 bp-long region of *HKR1* starting at the nt. #3050 through the ATG (^{3409}ATG) which corresponds to the internal translation initiation site (^{1137}Met). A significantly lower

transcriptional activity was detected when the reporter genes were ligated to the region between the nt. #2600 and ^{3409}ATG. These results suggest that the region between the nt. #3050 and the nt. #3330 is the core of the *HKR1* exonic promoter and its upstream sequence functions as a silencer-like regulator. Interestingly, the suppressed transcription was partially restored when the transformants were cultured under high osmotic pressure conditions.

These observations suggest a novel mechanism of eukaryotic gene regulation with multiple promoters, one of which is even located within an exon and is conditionally activated to express a latter portion of the protein. It could be a remarkable example of how a limited number of genes can generate more complex biological phenomena.

1364F **ChIP'ing away at Set1 regulation during meiosis** Johanna Maioriello, Michael Law Stockton University

Temporally and spatially restricted gene expression programs are required for differentiation. For example, in the budding yeast Saccharomyces cerevisiae, meiosis-specific genes are repressed during mitosis and must be expressed in waves of transcription, generally termed early, middle, and late. Transcriptional regulation relies upon extensive interactions between the RNA polymerase holoenzyme complex and post-translational histone modifications. The holoenzyme is comprised of multiple, highly-conserved protein subunits that include the cyclin C/Cdk8 kinase module. Previous studies have demonstrated functionally distinct structural and catalytic roles for Cdk8 in regulating gene expression, suggesting that complex mechanisms are in place for transcription during differentiation. Histone H3Lys4 methylation, catalyzed by the Set1-containing COMPASS complex, is one of the most well characterized of the histone modifications. While many studies have focused on determining the role of specific H3Lys4 methylation levels in controlling gene expression, less is known about how Set1 is targeted during differentiation. Our recent publications indicate that locus-specific Set1 recruitment and H3Lys4 me are antagonized by cyclin C/Cdk8 but the mechanism of this antagonism remains unknown. The goal of this study is to identify the spectrum of loci demonstrating meiosis-specific, Cdk8-dependent Set1 inhibition. To identify loci that are subject to control by Cdk8, we will use ChIP-seq. In these experiments, we will compare Set1 occupancy and activity in three different yeast strains; wild-type, cdk8Δ or cdk8 catalytic mutants, cultured in three different conditions; vegetative, pre-meiotic, or meiotic. Following crosslinking and chromatin fragmentation, Set1 occupancy will be measured using immunoprecipitations directed towards myc-Set1. Similarly, Set1 activity will be measured using immunoprecipitations directed towards histone H3Lys4 me1, me2, or me3. Next generation sequencing on purified DNA will be performed with the Illumina platform which will identify Cdk8- and growth condition-dependent Set1 regulation. In the wild-type strain, we will identify the regulatory pattern for meiosis-specific Set1 binding and H3Lys4 methylation. In our mutant strains, we will distinguish between the structural and catalytic functions of Cdk8. These studies will identify consensus promoter motifs regulated by Cdk8 and Set1 that are important for efficient meiotic transcription.

1365F **Characterization of histone mutants associated with recently described human neurodevelopmental disorders using the yeast model system** Agustin Kalinowski, Lillian Francis, Joseph Beard, Reece Forrest, Will Griffin, Jackson Parks, Caroline Tackett, Andrea Duina Department of Biology, Hendrix College

FACT is a highly conserved complex that plays critical roles in various chromosomal processes, including transcription elongation. During elongation, the histone chaperone complex FACT, which in *S. cerevisiae* is composed of two proteins, Spt16 and Pob3, contributes to the disassembling of nucleosomes in front of Pol II and their reassembly following Pol II passage. In previous work, we identified a nucleosomal region, which we refer to as ISGI (Influences Spt16-Gene Interactions), that plays a role in ensuring proper interactions between yeast FACT (yFACT) and transcribed genes. More specifically, mutations within this region cause an accumulation of yFACT at the 3' ends of genes, an effect we attribute to impaired yFACT dissociation following transcription.

Two recent studies have unveiled an association between mutations within genes that encode the human histone H3.3 protein and newly described neurodevelopmental disorders in patients. One of these mutants, H3.3 L61R, is within the ISGI region, providing a possible link between the neurodevelopmental disorders and yFACT-gene disassociation defects. To further explore this potential link and to gain a more general understanding of the effects of human H3.3 mutants on chromatin biology, we generated yeast cells that express several of these H3.3 mutants and we are currently testing them for possible defects in yFACT-gene interactions. We are also assaying the mutant cells for phenotypes indicative of nucleosome reassembly defects, DNA replication, and DNA repair. In this poster, we report our progress on these projects and describe our experimental plans moving forward.

1366F **Laboratory evolutions lead to reproducible mutations in *PDR3* conferring resistance to MCHM** Michael Ayers[1,2], Taizina Montareen[3], Daniel Judge[2], Dionysios Patriarcheas[2], Set Poziviak[2], Griffen Leombruno[2], Makaela Quinn[2], Camryn Lowery Lowery[2], Sarah McCulloch[2], Nathan Dale[2], Liam McCarthy[2], Felix Jonas[4], Jen Gallagher[2] [1]University of Rochester, [2]West Virginia University, [3]Biology, West Virginia University, [4]Constructor University

The solubility of protein complexes and membraneless compartments is maintained by liquid-liquid phase separation (LLPS). Phase transition is induced or dissolved by biological hydrotropes such as ATP and RNA. 4-methylcyclohexane methanol (MCHM), an alicyclic alcohol, is a synthetic hydrotrope that induces a starvation response by upregulation of biosynthetic pathways despite the availability of nutrients. To investigate how cellular metabolism can tolerate changes in LLPS, we evolved eight MHCM-resistant strains of *S. cerevisiae*. Knockouts of mutated genes were generated in the ancestral and evolved strains. These genes encoding proteins in flocculation, glutathione and SAM metabolism, and sugar transport and they mildly affected growth in MCHM. Among coding variants in the strains that change protein sequence and thereby may alter function, only one gene showed a protein-coding mutation in every resistant strain while showing no variants at all in the control strains. This gene, *PDR3*, controls transcription for the pleiotropic drug response and is the most significant driver of adaptive MCHM resistance in yeast. While many of the evolved alleles of *PDR3* would likely produce functional proteins, a knockout in the ancestral strain, YJM789, was sufficient to produce resistance to MCHM. Normal catabolism of amino acids uses the Pleiotropic Drug Response (PDR) pathway to export breakdown products and amino acid levels are elevated in MCHM exposed yeast. The *pdr3* resistance is mediated through Med15, a component of the Mediator complex which regulates activation by transcription factors of RNA pol II. Med15 contains two polyQ tracts that will transition into LLPS during stress and variation in length of the tracts changes the cellular response to MCHM. Pdr3 can homodimerize or dimerize with Pdr1, another transcription factor and loss of Pdr1 also confers MCHM resistance. Mutations in *PDR3* are first known to increase resistance to this novel hydrotropic chemical.

1367F **Flipping the script: How meiotic transcription controls the Set1 histone methyltransferase** Yasmin Mourad, Jayne E Seitz, Michael Law Stockton University

In all sexually-reproducing organisms, meiosis is a specialized cell division that is characterized by one round of DNA replication, homologous pairing and recombination, and two rounds of division (meiosis I and II) to give rise to four genetically distinct haploid gametes. This process requires faithfully executing a meiosis-specific gene expression program. In the budding yeast, Saccharomyces cerevisiae, three genes, IME1, IME2, and NDT80, are central components of the meiotic transcriptional cascade. IME1 is required for meiotic entry, IME2 coordinates multiple steps during early meiosis including homologous recombination and NDT80 activation, and NDT80 is required for cell commitment to divide. Previous work by our lab and others demonstrated that the histone H3Lys4 methyltransferase, Set1, is required for early meiosis with a critical role in genetic recombination, but that it represses transcription of a subset of Ndt80-dependent genes. These data indicate that Set1 inactivation is a critical factor for cells to commit to meiosis. Consistent with this model, we have recently found that Set1 is degraded during meiosis, suggesting that this is a key molecular switch for meiotic progression. Here we aim to identify how Set1 degradation is integrated into the meiotic transcriptional program. In this study, we combine genetic deletion mutations and conditional alleles to determine the requirement of IME1, IME2, and NDT80 in triggering Set1 degradation. To do this, we will measure Set1 protein levels during meiosis in three yeast mutant strains. The first strain harbors an ime1Δ mutation, which will allow us to determine the requirement of meiotic induction for Set1 degradation. Similarly, we have generated yeast harboring ime2 analog sensitive alleles. When grown in the presence of the ATP analog, 1-NA-PP, Ime2 activity is selectively inhibited. This will allow us to pinpoint when Ime2 kinase activity is required for Set1 degradation. Finally, we will utilize an estradiol-inducible form of NDT80. Yeast harboring this promoter will arrest prior to MI in the absence of beta-estradiol and will synchronously transition through meiotic commitment upon its addition. These experiments will determine the requirement of NDT80 expression and meiotic commitment in the Set1 degradation process. Together, these data will allow us to understand how Set1 activity is required for progression through recombination and meiotic division.

1368F **Using Budding Yeast to Model and Characterize Human Oncohistone Mutants** Celina Y Jones[1], Kirti Sad[2], Milo B Fasken[1], Jennifer M Spangle[2], Anita H Corbett[1] [1]Biology, Emory University, [2]Radiology Oncology, Emory University

Packaging genetic material into the nucleus is a major challenge that all eukaryotic organisms face. This packaging is accomplished by wrapping the genome around histone proteins. Hence, histones control chromatin accessibility and gene expression, which are largely regulated by histone post translational modifications (PTMs). Recently, recurrent missense mutations in genes encoding histones were found to drive oncogenesis in humans, creating oncohistones. Understanding how these pathogenic missense mutations alter histone function is challenging in humans as the four core histone proteins are each encoded by 12 – 15 genes, while budding yeast only contain two genes encoding each protein. Thus, engineering genetically homogenous strains that solely express the oncohistone mutant of interest is less technically challenging in yeast, facilitating clean characterization of altered PTMs and downstream mutant-driven molecular disruptions. To more faithfully model the dominant effects observed in humans, yeast can be engineered to express both a mutant and wild type histone copy. Our group has leveraged the strengths of budding yeast to model and characterize known and putative oncohistone mutants. We previously performed a high copy suppressor screen to identify suppressors of growth defects in histone H3K36 mutant cells, which model the oncohistone H3K36M. This study revealed connections to histone modifying enzymes, with investigations underway to define the mechanisms of suppression. Additionally, modeling putative oncohistones in yeast is

useful for characterizing the mechanism of action of these new mutations. The H3K36 mutant suppressors were expressed in the putative oncohistone H3E50K and revealed minimal suppression of growth defects, suggesting a different mode of molecular disruption as compared to H3K36 mutant cells. The H3E50K cells also displayed growth sensitivity when exposed to cellular stress and induced DNA double strand breaks. An added benefit to studying these mutations in yeast is how amenable this model organism is to undergraduate research, enabling us to prepare young scientists for careers in biological research. Employing yeast to investigate oncohistones promotes rapid discovery in disease-driving mutation research.

1369F **Investigating the function for the yeast lysine methyltransferase Set6 in proteostasis** Luke Mason, Erin Green, Deepika Jaiswal University of Maryland Baltimore County

Post-translational modifications (PTMs) of proteins facilitate control over cellular processes such as signaling cascades, allowing the cell to respond to changing environments. Methylation of lysine residues is a key regulatory mechanism that controls protein function. Lysine methyltransferases catalyze methylation on target proteins in response to signaling cues. One subset of lysine methyltransferases are the SMYD family of enzymes, which are well conserved and implicated in muscle development and differentiation in mammals. One of the SMYD enzymes in *Saccharomyces cerevisiae* is known as Set6, however, very little is known about its biological functions, catalytic activity, and protein-protein interactions. While lowly expressed under standard conditions, Set6 is upregulated during stress, such as nitrogen starvation. Based on phenotypes of *SET6* mutants, we have uncovered a role for Set6 in the proteostasis network, particularly autophagy. Proteomic screens have identified potential interaction partners of Set6 such as the GimC/Prefoldin complex and the Hsp70 co-chaperone Ydj1. Further proteomic screens have also identified candidate substrates for Set6 methyltransferase activity. Altogether, we identified the first known functions of the orphan lysine methyltransferase Set6 and characterized it as a new regulator of proteostasis.

1370F **Genetic analysis of the epigenetic regulation of liver development using a novel transgenic zebrafish epigenetic reporter line** Miranda Marvel, Yehyun Abby Kim, Kiyohito Taimatsu, Daniel Castranova, Allison Goldstein, Aniket V Gore, Brant M. Weinstein NICHD/NIH

Despite the critical importance of epigenetic mechanisms such as DNA and histone methylation in gene regulation and cell and tissue differentiation during development, most vertebrate tissue-specific epigenetic regulators are still unknown. Large-scale genetic screens performed in fruit flies and nematode worms have been very successful in identifying epigenetic regulators in invertebrates, but comparable screens have not been carried out in vertebrates. Our lab recently developed a novel “EpiTag” zebrafish transgenic epigenetic line that reliably reports changes in tissue-specific epigenetic silencing based on the dynamic expression of destabilized green fluorescent protein (GFPd2). We used this line to perform the first large-scale forward genetic screen for epigenetic-related mutants in a vertebrate, uncovering several mutants with persistent EpiTag reporter activation in the liver. We mapped one of these mutants to *ppat,* a gene coding for a phosphoribosyl pyrophosphate amidotransferase involved in purine nucleotide biosynthesis. The *ppat* gene is highly and specifically expressed highly in the liver, and CRISPR/Cas9-mediated targeting of the *ppat* locus phenocopies the liver activation of the EpiTag transgene. Surviving mutant animals develop dysfunctional, fatty livers. Preliminary results reveal transcriptomic and epigenetic changes in the livers of *ppat* mutants compared to wild-type animals that we are further characterizing in order to understand how epigenetic changes linked to *ppat* control critical liver development pathways.

1371S **A SID-1-dependent gene within a retrotransposon enhances heritable RNA silencing** Aishwarya Sathya, Nathan Shugarts, Andrew Yi, Antony Jose University of Maryland

RNAs in circulation carry sequence-specific regulatory information between cells in animal, plant, and host-pathogen systems. Such extracellular RNAs can function across generational boundaries in *C. elegans*. Double-stranded RNA (dsRNA) from neurons or the body cavity can enter the cytosol through the dsRNA importer SID-1 and silence genes of matching sequence in the germline and in progeny. Here we show that such SID-1-dependent regulation reduces heritable RNA silencing by downregulating a gene located within a retrotransposon. Using RNA-seq of wild-type, *sid-1(-)* mutant, and reverted *sid-1(+)* animals, we identified a *sid-1*-dependent gene *sdg-1*, which is located within a copy of the Cer9 retrotransposon. Changes in SDG-1 expression upon loss of SID-1 can last for more than 100 generations after SID-1 is restored. Perturbations of RNA-mediated regulation within the germline result in opposite effects on *sid-1* and *sdg-1*. Animals that lack SID-1 or that overexpress SDG-1 both show enhanced initiation of heritable RNA silencing upon mating. Furthermore, the SDG-1 protein is abundant within the germline and colocalizes with perinuclear condensates called Z granules, which are required for heritable RNA silencing. Since such silencing targets retrotransposons, these results suggest an auto-inhibitory loop that limits silencing of the *sdg-1*-containing retrotransposon. Intriguingly, SDG-1 (encoded by *F07B7.2/W09B7.2*) has two other paralogs *ZK262.8* and *C03A7.2*, both of which are encoded by genes located within Cer8 retrotransposon sequences. Furthermore, loss of *ZK262.8* has been reported to be synthetic lethal with loss of the miRNA-associated Argonaute ALG-2. Therefore, we speculate that inclusion of genes that regulate RNA silencing enables some retrotransposons and

retrotransposon-derived sequences to persist over evolutionary time.

1372S **Functional Characterization of systemic RNA interference in *C. elegans*** Faith Akoachere[1], Sarah E. Hall[2] [1]Syracuse University, [2]Biology Department, Syracuse University

Environmental stress during critical periods of development can result in altered gene expression and phenotypes in adulthood, which are sometimes inherited over generations. However, the exact mechanisms establishing and maintaining changes in gene expression due to environmental stress are not well understood. We showed previously that *C. elegans* hermaphrodite adults that had transiently passed through the dauer diapause stage due to crowding (postdauers) had significantly more progeny than continuously developed adults. Interestingly, this phenotype required the transmembrane channel protein SID-1 (systemic RNA interference defective). SID-1 is a vital effector of systemic RNAi (RNA interference) because it imports double-stranded RNAs (dsRNAs) into cells and signals the onset of gene silencing. To investigate mechanisms of endogenous systemic RNAi, we used a strain that carries a pan-neuronal, extrachromosomal *rab-3p::mut-16* overexpression transgene that results in progressive sterility over several generations. Additionally, hermaphrodites that have lost the extrachromosomal transgene continue to exhibit significantly reduced progeny numbers for three generations. We hypothesized that the overexpression of MUT-16 triggers the overproduction of small interfering RNAs (siRNAs) in neurons that are transported to the germ line resulting in aberrant gene expression that negatively affects reproduction. To test this hypothesis, we examined the transgenerational progeny number of animals carrying the *rab-3p::mut-16* overexpression transgene in a *sid-1* mutant background. Our results showed that SID-1 is not required for the transgenerational sterility phenotype, suggesting that MUT-16-dependent siRNAs in neurons are affecting germline function through a different mechanism. To determine how SID-1 is required for increased progeny number in postdauer adults, we are generating a strain with auxin-induced degradation and 3xFLAG epitopes inserted at the endogenous *sid-1* locus using CRISPR/Cas-9 genomic editing. We will attempt to immunoprecipitate SID-1 from control and postdauer adults, followed by RNA-seq to identify endogenous RNAs that pass through the channel to regulate gene expression across cell and tissue types. Together, these experiments will further characterize the role of SID-1 and endogenous RNAi in the regulation of developmentally programmed gene expression in C. elegans.

1373S **A major regulator of germline transcription, LSL-1, contributes to developmental defects when histone methylation is inappropriately inherited** Benjamin Nguyen, Brandon Carpenter Kennesaw State University

Histone methylation is a post-transcriptional modification to the N-terminal tails of histone core proteins that regulates DNA accessibility, and consequently, gene expression. Like DNA, histone methylation can be inherited between generations, and is highly regulated during embryonic development. At fertilization, histone methylation must undergo maternal reprogramming to reset the epigenetic landscape in the new zygote. During maternal reprogramming of histone methylation in the nematode, C. elegans, H3K4me (a modification associated with active transcription) is removed by the H3K4 demethylase, SPR-5, and H3K9me (a modification associated with transcriptional repression) is subsequently added by the histone methyltransferase, MET-2. Maternal loss of SPR-5 and MET-2 results in ectopic expression of germline genes in somatic tissues and a range of developmental phenotypes, including a severe developmental delay. Using a combination of RNA-seq and ChIP-seq experiments, a recent study identified a major regulator of germline transcription, LSL-1, that binds and turns on germline genes in the germline during development. From our own transcriptional analysis performed on C. elegans lacking SPR-5 and MET-2, we find that lsl-1 is significantly upregulated in somatic tissues. Together these data suggest that LSL-1 may be turning on germline genes aberrantly in somatic tissue and contributing to developmental delay. To test this hypothesis, we knocked down lsl-1 using RNA interference (RNAi) and found that the developmental delay in spr-5; met-2 mutants is significantly rescued. Using RNA-seq, we further demonstrate that knocking down LSL-1 in spr-5; met-2 mutant rescues ectopic expression of MES-4 germline genes. Together, our findings provide mechanistic insight into how inappropriate inheritance of epigenetic states perturb germline versus somatic cell fates specification during development and how this perturbation contributes to developmental phenotypes.

1374S **Selecting genes for analysis using historically contingent progress: RNA silencing in *C. elegans* as a case study** Farhaan Lalit[1], Antony Jose[2] [1]University of Maryland, [2]Cell Biology and Molecular Genetics, University of Maryland

Progress in biology has generated numerous lists of genes that share some property. However, detailed analysis of the genes is often required to discern mechanism(s) that could explain a list. With limited time and resources, prioritizing such regulated genes for detailed analyses is a key challenge faced by individual research labs and entire fields. Here we report a general approach for comparing tables of data accumulated by a field to identify genes of interest for further study. We apply this approach to RNA silencing in *C. elegans* and identify genes that appear to be selectively regulated but are understudied. We compared ~500 lists of genes reported in 113 studies as having significant changes in mRNA or antisense small RNA, or as encoding proteins interacting with a regulator of RNA silencing. The most selectively regulated genes or those encoding

proteins that interact with selective complexes were identified by their presence in shorter lists. Extent of co-occurrence of such regulated genes across studies was captured as historical mutual information (HMI) and used as a quantitative measure of the partially subjective links between genes revealed by the studies. Clustering the top 100 selectively regulated genes based on their HMI scores revealed three clear communities. Better studied genes were together in one cluster, suggesting that choosing other genes in that cluster for detailed analysis could also be illuminating. Most other genes in this cluster encode proteins reported as physical interactors of proteins that had been selected for study by the field, likely reflecting the need for study before being able to identify interacting partners. Intriguingly, many of these interactors have been implicated in other processes, providing regulatory links between RNA silencing and processes like the cell cycle. The other two clusters include genes and pseudogenes that have been described as targets of RNA regulation. These genes, which are present in many lists, could be regulated by multiple regulators independently and/or be jointly regulated by a connected set of regulators, making them potentially general sensors of perturbation in RNA silencing. These insights suggest that a similar analysis of genes in any field could aid progress from the initial implication of a set of genes in a process to understanding their roles in the process.

1375S **The relationship between period protein homolog LIN-42 and the conserved kinase KIN-20** Eileen McCleary[1], Collin Parrow[1], Benjamin Godbout[1], Joan Jatto[1], Michelle Coluzzi[1], Katherine McJunkin[2], Priscilla Van Wynsberghe[1] [1]Biology, Colgate University, [2]NIDDK NIH

The *C. elegans* heterochronic pathway, which regulates developmental timing, is thought to be an ancestral form of the circadian clock in other organisms. An essential member of this clock is the Period protein whose homolog, LIN-42, in *C. elegans* is an important regulator of developmental timing. LIN-42 functions as a transcriptional repressor of multiple genes including the conserved lin-4 and let-7 microRNAs. Like other Period proteins, levels of LIN-42 oscillate throughout development. In other organisms this cycling is controlled in part by phosphorylation. KIN-20 is the *C. elegans* homolog of the Drosophila Period protein kinase Doubletime. Worms containing a large deletion in kin-20 have a significantly smaller brood size, develop slower than wild type *C. elegans*, and display an uncoordinated phenotype. We have previously shown that KIN-20 impacts *lin-42* phenotypes. In addition, KIN-20 is important for post-transcriptional regulation of mature let-7 and lin-4 microRNA expression. However, the mechanisms by which KIN-20 regulates LIN-42 and microRNA biogenesis are unclear. Using epitope-tagged variants of LIN-42 and KIN-20, we have found that KIN-20 impacts LIN-42 expression. Current work aims to determine how KIN-20 causes these effects by analyzing phosphorylation target mutants of LIN-42 and a KIN-20 mutant deficient in kinase activity. Altogether, these findings further our understanding of the mechanisms by which these conserved circadian rhythmic genes interact to ultimately regulate rhythmic processes and developmental timing in *C. elegans*.

1376S **The effect of environmental factors on core circadian clock genes in *C. elegans*** Carson Hobler, Bronwen Rees-Wiedemann, Julia Shavo, Mason Caldwell, Kendall Mueller, Jenna Borovinsky, Michelle Coluzzi, Priscilla Van Wynsberghe Biology, Colgate University

Circadian rhythms are entrained by environmental cues like light and typically follow a 24-hour cycle. These rhythms are dictated by circadian clock genes and are vital for regulating internal functions and processes. Central to the circadian clock is the Period protein, a transcription factor whose levels oscillate. *C. elegans* exhibit many rhythmic behaviors and express some of the core circadian clock genes. However, limited information is available regarding how environmental factors like light impact *C. elegans* core clock genes. LITE-1 and GUR-3 are both 7-transmembrane gustatory receptors in *C. elegans*. Though LITE-1 lacks a typical chromophore, it is important for detecting both UVA and UVB light, and can sense and mediate responses to high concentrations of hydrogen peroxide. Meanwhile GUR-3 is able to distinguish small changes in hydrogen peroxide at lower concentrations. In addition to sensing these external factors, LITE-1 and GUR-3 are also critical for the synchronization of *C. elegans* behavior to daily light cycles and are required for light-induced avoidance behavior. Here we sought to understand the role of LITE-1, if any, in regulating the core circadian Period protein, LIN-42, in *C. elegans*. Since LITE-1 is important for UV light avoidance, we first analyzed light avoidance phenotypes in young adult *lin-42* and *lite-1* mutant worms. As expected, WT N2 worms migrated away from the light stimulus while *lite-1* mutant worms demonstrated less UV light avoidance. Interestingly, both *lin-42* and *lite-1;lin-42* mutant worms exhibited less UV light avoidance than *lite-1* mutant worms. We also found that when directly exposed to UV light, *lin-42* mutant worms exhibited more paralysis compared to WT N2. This suggests that LIN-42 plays some role in the photosensation pathway. Analysis of LIN-42 protein levels in *lite-1* mutants by western blotting shows that LITE-1 impacts LIN-42 protein expression. We further find that PRDX2, an antioxidant enzyme peroxiredoxin that alters behavior in response to hydrogen peroxide, impacts expression of the conserved miRNA let-7. Current work aims to better understand how LITE-1 and GUR-3 impact core circadian clock genes and the LIN-42 target let-7. Altogether, this work will help us understand the mechanism by which *C. elegans* core clock genes respond to environmental cues.

1377S **HIF-1c, an oxygen-insensitive HIF-1 isoform in *C. elegans,* is expressed tissue-specifically via an internal promoter.** Amir Sabeti Aghabozorgi[1], Kate Locsin[1], Chelsey Torres[1], Carlos Egydio de Carvalho[2] [1]Biology, University of

Saskatchewan, [2]University of Saskatchewan

Hypoxia-Inducible Factor-1 (HIF-1) is a conserved protein family of transcription factors in metazoans that evolved to cope with hypoxic stress by activating the expression of genes required for adaptation to low oxygen levels. Among animal cells, neurons are particularly susceptible to hypoxia-induced damage. Thus, unraveling how neurons differentially mobilize HIF-1 to cope with hypoxia is of clinical relevance. We identified a shorter *hif-1* transcript (*hif-1c*) in *C. elegans* with extensive expression almost exclusively in the nervous system. Aside from neurons, only a few muscle cells in the head region, the distal tip cell (DTC) in the somatic gonad and the two neuroendocrine uv1 cells express *hif-1c*. Using reporter lines, we identified and mapped a region within intron 4 of the *hif-1* locus that acts as an internal promoter to drive *hif-1c* transcription. Similar to the canonical HIF-1 isoform (HIF-1a), *hif-1c* predicts a protein unstable at room air, but unlike HIF-1a, without DNA binding activity. Expression and phenotypic characterization so far suggest that HIF-1c indeed cannot transactivate *hif-1* target genes, but it is unexpectedly oxygen-insensitive, despite its predictive oxygen-dependent degradation domain. Surprisingly, we find HIF-1c::GFP localizing specifically at the basal body of cilia of labial sensory neurons that control several behavioural responses in *C. elegans*. We are currently exploring possible sensory roles for HIF-1c in neurons.

1378S **Chemotaxis defect and developmental delay phenotypes in *spr-5;met-2* mutants may be caused by the ectopic expression of meiosis genes in somatic tissues.** Rhea Rastogi, Monica Reeves, Jaely Chavez, Juan Rodriguez, David J Katz Department of Cell Biology, Emory University

The proper formation of a zygote requires extensive epigenetic reprogramming to enable appropriate inheritance of histone methylation. In *C. elegans*, this reprogramming is partly mediated by two enzymes: H3K4me2 demethylase SPR-5/LSD1/KDM1A and H3K9 methyltransferase MET-2/SETDB1. Progeny of mutants lacking both SPR-5 and MET-2 suffer from a severe chemotaxis defect, L2 developmental delay, and sterility. These phenotypes are caused by the ectopic expression of germline genes in somatic tissues due to a synergistic increase in H3K4me2. Previously, we found that *spr-5* and *met-2* single mutants each have a transgenerational accumulation of H3K4me2, resulting in a germline mortality phenotype across generations. To determine if the chemotaxis defect in *spr-5;met-2* double mutants might be caused by H3K4me2 accumulation, we asked whether *spr-5* and *met-2* single mutants have a decrease in chemotaxis across generations. Although *spr-5* and *met-2* single mutants have a slight chemotaxis defect, it does not change across generations and is far less severe than that of *spr-5; met-2* double mutants. This suggests that the severe chemotaxis defect in *spr-5; met-2* mutants requires the loss of both enzymes. However, the lack of a severe chemotaxis defect in the single mutants provides an opportunity to identify molecular pathways underlying the behavioral defect in the double mutant by identifying gene expression changes that occur in the double mutant but not the single mutants. Thus, we performed RNAseq on L4 *spr-5* and *met-2* single mutants at F7, F13, and F22, from which we identified 460 genes that are uniquely upregulated in *spr-5; met-2* mutants. Gene ontology analysis indicates that this gene set is enriched in meiotic genes, raising the possibility that it is specifically the inappropriate expression of meiosis genes in somatic tissues causing the chemotaxis defect and developmental delay. Consistent with this, we find in *spr-5; met-2* mutants that while germline genes are ectopically expressed beginning in the embryonic stages, the meiotic genes are only ectopically expressed later in development when the chemotaxis defect and developmental delay are observed. Intriguingly, patients with neurodevelopmental disorders caused by mutations in histone-modifying enzymes also have meiotic genes ectopically expressed in the nervous system. Thus, it is possible that we have identified a conserved mechanism. We are currently pursuing this possibility using an LSD1 mouse model.

1379S **Characterization of NHR-25 genome-wide binding reveals role for combinatorial transcription factor action** Alexander Sinks, Leah Flautt, Kimberley Tanatswa Muchenje, Belle Ange Itetere, Deborah M Thurtle-Schmidt Biology, Davidson College

Terminal cellular differentiation and maintenance into distinct cell types is due in part to sequence-specific transcription factors (TFs) directing proper transcriptional regulation. TFs bind to response elements, establishing the proper gene regulatory network for that cell type. Thus, binding site recognition and subsequent regulation of the TF target gene to establish and maintain the correct gene regulatory network is critical for proper genomic functioning. However, how a TF recognizes its binding site and subsequent target genes remains poorly understood. *C. elegans* provides an excellent model to study transcriptional regulation due to its compact genome and highly conserved TFs. To investigate how a transcription factor identifies its binding site to target the correct target gene we profiled genome-wide binding of an endogenously tagged, highly conserved TF, NHR-25, using ChIP-seq and CUT&RUN in L1 worms. We identified 1140 NHR-25 enriched sites. 78% of these sites overlapped with previous NHR-25 ChIP-seq of a multi-copy integrant NHR-25 from the modERN resource, however the multi-copy integrant resulted in 5x as many enrichment sites, suggesting TF expression level is critical for binding. Motif analysis of NHR-25 occupied regions identified 23 enriched motifs, including the presumed NHR-25 binding site and a GAGA motif like the EOR-1 binding motif. Correlation with *C. elegans* chromatin domains (Evans et al 2016) showed enriched regions containing the NHR-25 motif at regions designated as enhancer-type chromatin. To identify the NHR-25 responsive genes and

correlate target genes to NHR-25 enriched regulatory elements, we performed RNA-seq of *nhr-25* knockdown worms and correlated differentially regulated genes to the nearest enriched peak. Many peaks were proximal to, but not directly adjacent to a differentially expressed gene. To directly correlate target genes of identified regulatory elements, we deleted NHR-25 enriched regions using CRISPR/Cas9 and profiled gene expression by RNA-seq. For a single deleted regulatory element, we identified two nearby genes with altered expression. However, only one of these adjacent genes showed NHR-25 dependent expression. Taken together, these data suggest combinatorial action of multiple TFs are required to direct proper gene regulation.

1380S **Characterizing the regulation of piRNA expression by the CSR-1 Argonaute** Victoria Murphy, Jessica Kirshner, Margaret Starostik, Sadhana Pani, John Kim Johns Hopkins University

In metazoans, germline gene expression is tightly controlled by small RNA pathways to promote germline development and animal fertility. The piRNA pathway suppresses foreign genetic elements, such as transposons, through base-pairing between the piRNAs and their target mRNAs. In opposition to this silencing pathway, a non-canonical endo-siRNA pathway, governed by the Argonaute, CSR-1, functions to promote expression of target genes in the germline. Together, the CSR-1 gene licensing pathway and piRNA-mediated gene silencing pathway tune germline gene expression. Despite the opposing functions of the CSR-1 and piRNA pathways, mechanisms of cross-regulation between them remain unknown. Here we show that CSR-1 suppresses the piRNA pathway through direct silencing of mRNAs encoding piRNA biogenesis factors. The piRNA biogenesis machinery is significantly enriched in the small subset of genes directly sliced by CSR-1. Therefore, in *csr-1* mutants, the piRNA biogenesis machinery becomes significantly upregulated during both spermatogenesis and oogenesis and is accompanied by the ectopic upregulation of piRNAs. Using small RNA sequencing, we observe a perdurance of spermatogenesis-specific piRNAs into oogenesis. Ongoing work will examine the impact of csr-1 defects on piRNA target silencing, chromatin defects at piRNA target loci, and defects in biogenesis of other classes of small RNAs resulting from elevated piRNA biogenesis factors. Finally, we are investigating the consequence of inappropriate expression of sex-specific piRNAs on germline development. Together, our data offer evidence of how gene licensing and silencing small RNA pathways are coordinated in *C. elegans* to regulate germline gene expression.

1381S **Identification of transcriptional regulators impacted by a glucose-supplemented diet in *C. elegans*** Jose Robledo, Mary Ladage, Rajeev Azad, Pamela Padilla Biological Sciences, University of North Texas

Type 2 diabetic patients display various metabolic changes (e.g. hyperglycemia, insulin resistance, increase in free fatty acids) that contribute to dysfunction in multiple organs. For example, hyperglycemia leads to permanent gene expression changes to organs that persist even after treatments regulate glucose levels. Genetics and environment play a huge role in the development and progression of type 2 diabetes. Change in gene expression is one underlying factor contributing to the physiological changes associated with diabetes. However, how factors such as hyperglycemia or a sugar-supplemented diet affect whole animal gene expression remains to be further explored. Our lab focuses on using the genetic model system *Caenorhabditis elegans* to study the impact a glucose-supplemented diet has on physiology, stress responses and gene expression. Previously, we showed that a glucose-supplemented diet impacts the expression of 2,370 genes, increases lipid accumulation, and increases sensitivity to stress. To further understand the gene expression differences due to a glucose-supplemented diet, we analyzed the transcriptomic data to identify potential glucose responsive genes. One such gene, the transcription factor acs-2, was then further investigated using qPCR and an analysis of an acs-2::GFP reporter strain. Here, we show acs-2 transcript to be a glucose-responsive gene. Additional bioinformatic analysis on our transcriptomic data also revealed 140 transcription factors (TFs) that were altered in response to a glucose-supplemented diet. In an effort to identify glucose responsive transcription factors, we developed a targeted RNA interference screen using the *acs-2::GFP* reporter strain. Our RNAi screen identified 45 transcription factors that significantly impact the expression of *acs-2* in a glucose diet dependent manner. We further classified our glucose-responsive TFs according to DNA binding domains and found 16 NHRs, 12 ZF, 5 bZip, 5 bHLH, and 7 unknowns TFs. Together, these studies add to our understanding of the molecular network that is impacted in response to a glucose supplemented diet.

1382S **Imprinting in *C. elegans*: maternal *sid-1* expression alleviates silencing of paternal *sid-1*.** Andrey Shubin, Craig P Hunter Harvard University

Genes transmitted by oocytes or sperm usually contribute equally to the development and function of progeny. An exception is imprinting in mammals, which is caused by gamete-specific DNA methylation patterns at targeted genes resulting in parent-of-origin effects on gene expression. Our studies identify a case of imprinting for the *sid-1* gene in *C. elegans*: crosses between wild-type males and *sid-1* deletion hermaphrodites produce only Sid progeny. Our analysis reveals that maternal *sid-1* mRNA or agents derived from it, are required to activate constitutively silenced paternal *sid-1.* This silencing is not observed in the progeny of *prg-1*-mothers or fathers, does not require chromatin-level interaction between alleles in the zygote for initiation or

maintenance of *sid-1* silencing, but simply depends on the absence of maternal *sid-1* transcripts. Furthermore, in the absence of an expressed allele, the silenced state can persist for four generations. Unlike the multi-generational silencing caused by the extrachromosomal *sid-1* promoter transgene array[1], which affects the entire locus (*sid-1* and two upstream genes), the paternal imprinting affects only the *sid-1* gene.

Our findings suggest a crucial role for maternal mRNA transcripts in the transcriptional activation or licensing of paternal genes in *C. elegans* that can otherwise be a subject to imprinting mediated by PRG-1-associated piRNAs. These results imply a regulatory interaction between transcriptional suppression mechanisms that protect against the expression of potentially harmful paternally derived alien genes or selfish genetic elements such as transposons and licensing of paternal genes by maternal mRNA.

1. Minkina O, Hunter CP. 2017. Stable Heritable Germline Silencing Directs Somatic Silencing at an Endogenous Locus. Molecular Cell. 65(4):659-670.e5. doi:https://doi.org/10.1016/j.molcel.2017.01.034.

1383S **Characterizing the miRNA Argonaute isoforms in C. elegans** James P Davis, Rima Sakhawala, Katherine McJunkin NIDDK, NIH

MicroRNAs (miRNA) are short, non-coding RNAs that regulate gene expression post-transcriptionally. MicroRNAs are loaded into an Argonaute protein which makes up the RNA-induced silencing complex (RISC) that leads to the repression of target mRNAs. A recent study focusing on the CSR-1 Argonaute has found that the csr-1 locus encodes two isoforms, CSR-1a and CSR-1b. These isoforms show tissue specific functions with CSR-1a regulating immunity and pathogen response genes while CSR-1b regulates spermatogenesis genes. In C. elegans, two of the miRNA Argonautes, ALG-1 and ALG-2, encode two isoforms. We are interested in examining if ALG-1 and ALG-2 have isoform-specific functions. We have fluorescently labeled the ALG-1 long isoform with mKate and crossed that into a strain that had both ALG-1 isoforms tagged with GFP. Heterozygotes from this cross were imaged to identify where the long isoform is expressed. We found that the long isoform is expressed in the head ganglia, ventral nerve cord, and tail ganglia. We have designed strains in which we mutated either the long or short isoform of ALG-1 and ALG-2. We are crossing these strains into an ALG-1(-/-) or ALG-2 (-/-) background to determine if a specific isoform alone is sufficient for development, since ALG-1 and ALG-2 are functionally redundant. When only the ALG-1 long isoform was expressed we observed bursting and protruding vulvas in the young adult population. We hypothesize that the long and short isoforms have different miRNA loading profiles, which may explain the mutant phenotype when only one isoform is expressed. We are currently in the process of generating the other strains to determine if they have differing phenotypes as well. Thus far, we conclude that there may be an isoform specific function between the ALG-1 long and short isoform, and further characterization is needed. Further study is needed to determine if there is isoform specific function between the ALG-2 long and short isoform as well.

1384S **Identification of the EOR-1 and NHR-25 shared transcriptome** Amanda Fuenzalida, Shawn An, Tiffany Haydt, Deborah M Thurtle-Schmidt Biology, Davidson College

Correct gene regulation is critical for establishing and maintaining correct cellular identity. Transcription factors (TF) are regulatory proteins that bind to motifs across the genome, regulating gene expression. In *C. elegans*, NHR-25 is an essential TF involved in somatic cell differentiation and gonadal development. ChIP-seq of NHR-25 revealed that in addition to NHR-25's own known binding motif, a GAGA motif was also enriched at NHR-25 associated sites in the genome. EOR-1 is a transcription factor in the Ras/ERK signaling pathway thought to act as both a potential activator and repressor. Previous literature in *C. elegans* and orthologs of EOR-1 in other organisms suggest that this transcription factor may act as a pioneer factor. Here we hypothesize that EOR-1 is a necessary pioneer factor for NHR-25 and coordinates correct gene regulation. To investigate this hypothesis, we profiled the transcriptome of *eor-1(cs28)* and *nhr-25(ku217)* mutants at two different developmental times (L1 and L3) and temperatures (20˚C and 25˚C). Differential expression analysis will identify shared regulated genes sets, revealing the potential extent of EOR-1's ability to act as a pioneer factor.

1385S **Exploring How Cells Randomly Choose Between Fates in the Fly Eye** Christina Im, Alison Ordway, Robert Johnston Biology, Johns Hopkins University

Development of an organism is complex and requires many different processes to occur simultaneously. Although many developmental processes are carefully regulated, some occur stochastically. A stochastic process refers to one that involves a random variable. These mechanisms diversify cell types and generate additional functions.

The fly eye provides an excellent model to study stochastic patterning during development. The *Drosophila* (fruit fly) eye consists of ~800 unit eyes, or ommatidia. Each ommatidium is composed of eight photoreceptors (R1-R8). The R7 photoreceptor has two distinct subtypes, defined by expression of light detecting Rhodopsin proteins: Rhodopsin 3 (Rh3) or

Rhodopsin 4 (Rh4). A stochastic choice between expression of these Rhodopsin proteins is controlled by the transcription factor *spineless* (*ss*). In Ss^{ON} cells, Rhodopsin 4 is expressed whereas in Ss^{OFF} cells, Rhodopsin 3 is expressed. In wild-type flies, *ss* is on in about 67% of R7s.

During development of the eye, photoreceptor specification occurs in a wave across the developing eye field. *ss* is expressed dynamically during development. This dynamic expression is controlled by three elements at the *ss* gene locus: the *promoter*, the *early enhancer*, and the *late enhancer*. The *early enhancer* drives early expression in all R7 precursor cells and the *late enhancer* drives late expression in a subset of terminally differentiated R7 cells, but how they regulate *spineless* remains poorly understood. My research project focuses on the role of the *late enhancer* in regulating *ss* expression during stochastic cell fate specification.

1386S **Modulation of tumor growth by Yorkie and Wingless** arushi rai[1], Amit Singh[2,3,4], Madhuri Kango-Singh[1,3,4] [1]Growth Regulation and Signalling Laboratory, Department of Biology, University of Dayton, [2]Drosophila Development and Disease Laboratory, Department of Biology University of Dayton, [3]Premedical Program, University of Dayton, [4]Integrative Science and Engineering Center, University of Dayton

Studies with *Drosophila* Ras^{V12}, $scrib^{-/-}$ tumor models have shown that Yorkie, the effector molecule of the Hippo pathway, interacts with other signaling pathways to form a dynamic transcriptional network within cancer cells. Previously, we have shown that in Ras^{V12}, $scrib^{-/-}$cells Wingless (Wg) acts upstream of Caspases, JNK, and Yki forming a tumor-specific network that regulates tumor growth and development. By studying this complex network, we aim to unravel the key players that regulate tumor growth. Wg appears upstream of the molecular network and is also a transcriptional target of Yorkie. Wg expression is ectopically induced in Ras^{V12} and Ras^{V12}, $scrib^{-/-}$ tumor clones. However, accumulation of Wg is reduced in tumor clones with both heterozygous loss of *yki* ($yki^{B5}/+;Ras^{V12},scrib^{-/-}$) or downregulation of Wg signaling ($dTCF^{DN};Ras^{V12},scrib^{-/-}$). Heterozygosity of *yki*($yki^{B5}/+;Ras^{V12},scrib^{-/-}$) and downregulation of Wg signaling ($dTCF^{DN};Ras^{V12},scrib^{-/-}$) both resulted in reduced tumor clone size in comparison to $Ras^{V12},scrib^{-/-}$ tumor clones. To further understand the molecular mechanism by which Wg and Yki promote tumor growth, we will investigate the effect of downregulation of Wg pathway and Hippo pathway on (a) the transcriptional network of $Ras^{V12},scrib^{-/-}$ tumor clones by analyzing the mRNA expression by qRT-PCR, (b) hallmarks of cancer such as invasion, cell adhesion, and survival signaling by immunohistochemistry-based approach, and (c) analyzing the Hippo pathway (Mst, p-MST) JNK pathway (JNK, p-JNK) and Wg pathway activity by Western blot assay. Here, we present our progress on the organization of the molecular network that involves Wingless and Yorkie.

1387S **Understanding the role of Embargoed in the specification of muscle fiber fates in *Drosophila*** Alexis Guzman, Richard Cripps Biology, San Diego State University

Understanding the mechanisms that lead to the specification of muscle fiber fates during Drosophila development remain unclear. The homeodomain proteins Extradenticle (Exd) and Homeothroax (Hth) are normally expressed in the adult fibrillar indirect flight muscles but not in the tubular jump muscles. Knocking down Exd and Hth converts flight muscles into jump muscles. Exd and Hth are initially expressed within the myoblasts of the jump muscle cells, but these proteins are no longer detected upon the initiation of myoblast fusion. We have thus sought to determine how the expression pattern of these proteins changes during development. Embargoed (Emb), which encodes an exportin involved in nuclear export, is proposed to play a role in transporting Exd and Hth out of the nucleus of the jump muscle cells. Indeed, knockdown of Emb expression using RNAi transforms the jump muscles into a flight muscle fate. We hypothesize that when Exd or Hth are phosphorylated in the flight muscle myoblasts, Emb cannot bind and export these proteins out of the nucleus. In the jump muscles, Exd and Hth are not phosphorylated by this kinase, which allows Emb to bind and transport them to the cytoplasm. By knocking down the kinase that phosphorylates Exd or Hth in the jump muscles via RNAi, we expect to see ectopic expression of Exd and Hth in the jump muscles after myoblast fusion, as well as a conversion to a flight muscle identity. To further confirm this, we will stain for flight and jump muscle markers to quantify the extent of this muscle fiber switch. The mammalian ortholog of Emb, Exportin 1 (XPO1), is also a protein that mediates the nuclear export of proteins and RNAs which suggests that a similar mechanism in the specification of muscle fiber fates may be conserved.

1388S **Effects of mutation in the P38 docking domain of MEF2 on its function** Bhumika Gode, Richard Cripps Biology, San Diego State University

MEF2, or Myocyte enhancer factor, is a conserved transcription factor with a critical role in human muscle gene expression, as well as Drosophila muscle development. Our study focuses specifically on Drosophila muscle development, which serves as a model organism due to its compact size, ease of maintenance, and suitability for research in resource-constrained laboratories. MEF2 plays a crucial role in differentiation of all muscle types including dorso-longitudinal indirect flight muscles (DLMs), jump muscles (TDT - tergal depressor of trochanter), and leg muscles. In mammalian tissue culture studies, P38, a mitogen-activated protein kinase (MAPK), enhances MEF2 activity by phosphorylating it via binding to a P38 docking site. Our study aims to

investigate how a mutation in the P38 docking site affects MEF2 function. Using CRISPR, we induced a 7-amino acid (or 21-base pair) deletion in the P38 domain of Mef2. We conducted immunostaining on longitudinal and transverse sections of mutant Drosophila thoraces to visualize the structures of jump muscles and DLMs, respectively. Additionally, we analyzed the relative gene expression of genes that are transcriptional targets of MEF2 like Actin 57B (Act57B), Myosin heavy chain (MHC), and Troponin I (TnI). Immunostaining revealed significant patterning defects in the jump muscle structure in mutant flies compared to wild-type, while there was no difference in the number of DLM fibers. We plan to perform mutagenesis experiments to examine the potential binding of human P38 to a mutated Drosophila MEF2 protein gene. We also want to further investigate additional significant effects of the mutation, particularly on their ability to jump and other developmental factors. These studies identify a conserved and functionally significant interaction between MEF2 and P38.

1389S **Analysis of chromatin modifications at *Dmef-2* enhancer during myogenesis** Sara Khadraoui[1], Sarah Anglin[2], Scott Nowak[2] [1]Kennesaw State University, [2]KSU

The nuclear transcription cofactor Akirin plays a key role in the regulation of *Dmef2* (Drosophila myocyte enhancer factor 2) during the early steps of embryonic myogenesis. Akirin is thought to help regulate *Dmef2* expression levels by mediating an association between chromatin remodeling complexes and Twist transcription factor activity. Previous work by the Nowak laboratory has determined that Akirin genetically and physically interacts with either the Brahma (SWI/SNF) chromatin remodeling complex, or through genetic interactions with the Nucleosome Remodeling and Deacetylase Complex. These interactions are essential for proper cardiac and skeletal patterning and development during embryogenesis. While both complexes have seemingly contradictory activities, the exact nature of covalent histone modifications that occur at *Dmef2* enhancers during myogenesis remains unknown. Using a variety of antibodies targeting various covalent histone modifications in chromatin immunoprecipitation we have begun an analysis of chromatin modifications that occur at the *Dmef2* enhancers during early, mid, and late embryonic myogenesis in both wild-type and *akirin* mutant backgrounds. Our results indicate that the histone modification landscape at the *Dmef2*enhancer is highly varied in *akirin* mutant backgrounds, which supports previous studies indicating that recruitment of chromatin remodeling complexes to these loci during myogenesis is key for their proper expression levels.

1390S **Unveiling the role of Hippo Interactors in Glioma Progression in Drosophila glioma model** Venkata Satya Devi Burugupalli[1], Madhuri Kango-Singh[2,3,4], Arushi Rai[1], Amit Singh[3,4,5] [1]Growth Regulation and Signalling Laboratory, Department of Biology, University of Dayton, [2]Growth Regulation and Signalling Laboratory, Department of Biology University of Dayton, [3]Premedical Program, University of Dayton, [4]Integrative Science and Engineering Center University of Dayton, [5]Drosophila Development and Disease Laboratory, Department of Biology University of Dayton

Signalling interactions play an important role in tumorigenesis. The Hippo signalling pathway originally identified for its role in organ size maintenance also plays a key role in tumor progression. Using the previously published Drosophila glioma model in which coactivation the EGFR receptor and Phospho-inositol 3 kinase (PI3K) pathways in the CNS glia using repoGAL4, we plan to study the role of interactions of Yorkie (YAP/TAZ in human), the downstream effector of the Hippo pathway, with other pathways in promotion of glioma growth. Given the conservation of genes and cell biological pathways, finding from our studies are expected to be extrapolated to higher vertebrates. Initial studies showed the role of Yorkie in neural stem cells in the growth of glioma. Here we present our progress with evaluating the role of other Yki interacting pathways using immunostaining, western blots and qPCR analyses to investigate which pathways are important for glioma progression.
Keywords: Hippo pathway, GAL4-UAS, Glioma and signalling.

1391S **Establishing the Role of the Conserved TN Domain in Tinman** Cayleen Bileckyj, Richard Cripps San Diego State University

Congenital heart disease (CHD) is a major factor in mortality and morbidity in children and adults. To improve our ability to identify and manage CHD, we need to further understand the key genetic factors involved in causing these disorders.

Drosophila melanogaster, a model organism with cardiac developmental mechanisms analogous to those that occur in human cardiac development, has been an essential vehicle in furthering our understanding of the proteins and pathways involved in cardiogenesis. Tinman (Tin), a transcription factor in *Drosophila* vital for cardiac cell differentiation, and its mammalian ortholog NK2 Homeobox 5 (Nkx2.5) share two conserved regions: the homeobox and the tin (TN) domain. Although the TN domain is completely conserved between these two proteins, there is little known about its significance.

The goal of my research is to establish how the TN domain impacts the ability of Tin to function during development. To do this, I observe how the removal of the TN domain from Tin effects expression of various proteins in cardial, pericardial, and precursor cardial cells during embryogenesis. Data generated for this portion has come from a line of *Drosophila* I generated using CRISPR/Cas9 gene editing that contain an in-frame deletion of the TN domain. My project also focuses on describing how the deletion of the TN domain effects Tinman's ability to physically interact with other proteins. Transfection of plasmids containing wild type Tin, Tin with the TN domain removed, and the suspected co-repressor Groucho (Gro) into Drosophila S2 cells, followed by co-immunoprecipitation, will elucidate if the TN domain is necessary for Tin and Gro to interact. Lastly, again using CRISPR/Cas9 gene editing, I established two fly lines that contain two different known mutations that occur in the TN domain in the human Nkx2.5 protein. Immunohistochemistry of these fly lines will reveal the impact these mutations have on Drosophila development, and therefore possible insight into how humans with these mutations could potentially be affected.

While the TN domain is completely conserved between Tinman and Nkx2.5, it can also be found partially conserved in other mammalian proteins involved in the different aspects of development. Therefore, not only can delineating the role of the conserved TN domain in Tin improve our understanding of cardiac formation and maturation, but it has the potential to inform upon other areas of developmental biology as well.

1392S **The role of epigenetic regulators in muscle specification during development** Elizabeth Barajas Alonso, Richard M. Cripps San Diego State University

Polycomb Group (PcG) proteins are chromatin modulators that have been shown to have an essential role in developmental processes by maintaining cell fate across different organisms. PcG proteins function as transcriptional repressors to remodel chromatin to a condensed state in which target genes become inaccessible from the transcriptional machinery. Furthermore, genetic screens in *Drosophila melanogaster* identified PcG proteins as regulators of *Hox* genes to ensure correct body patterning. Notably, de-regulation of PcG proteins has implications in several congenital disorders and cancers however, the role of these epigenetic regulators in muscle diseases and development, such as fiber type specification, has not been fully elucidated. We, therefore, seek to determine the effect of PcG loss of function upon muscle identity in *Drosophila.* To test this, we performed an RNAi screen of members in the Polycomb Repressive Complex (PRC) 1 and 2 using tissue specific drivers to target expression of the PcG RNAi in the flight and jump muscles, respectively. The screening revealed that expressing RNAi for *P55*, *Pcl*, *Sce*, and *Scm* resulted in a partial transformation of the flight muscles to a jump muscle fate. This was evidenced by a change in the expression of the fiber-specific reporter *TpnC41C-lacZ* in which expression expanded into the knockdown flight muscles. In addition, we also saw an altered myofibril striation pattern in the knockdown flight muscles where the z-discs appeared to be stacked, indicative of a tubular identity. Overall, our preliminary data suggests that PcGs play an essential role in regulating flight muscle fate.

1393S **Dude, where's my locus? Alternative methods of genomic targeting for specialized transcription factors.** Thomas OHaren, Skye Comstra, Leila Rieder Biology, Emory University

While many transcription factors recognize consensus DNA motifs and target many genomic loci, some are extremely specialized and bind only a single or few regions of the genome. Additionally, some specialized transcription factors do not bind or recognize DNA sequence, so it remains unclear what cues allow effective targeting of specialized factors to their regions of activity. One such factor is Multi sex combs (Mxc), a scaffolding protein of the Histone Locus Body (HLB), a collection of factors that assist in the specialized regulation and processing of histone mRNAs. The Drosophila melanogaster genome contains a single histone locus harboring 100+ copies of the canonical, replication-dependent histone genes, and Mxc is one of the earliest localizing factors to the locus. Many factors of the HLB are unique to histone gene regulation and target only the singular locus. However, none of the HLB-specific factors, including Mxc, bind DNA, and such, how the histone genes are identified in early development remains unknown. A deficiency Drosophila line in which the entirety of the histone locus is removed is heterozygous viable. Interestingly, Mxc and HLB factor FLASH continue to colocalize and form nuclear puncta in the fraction of homozygous early embryos that lack the histone genes. We hypothesize that the regions of the genome where HLB factors are misrecruited will reveal clues about what contributes to the identification of the locus and more generally how transcription factors behave when their target locus is removed. Genomic cues such as histone marks, chromatin state, and non-specific transcription factors could contribute to localization of extremely specialized factors that do not bind DNA. By utilizing Dam-ID and fusing a Dam (DNA adenine methyltransferase) domain to Mxc to circumvent the lack of direct DNA binding, we are labeling regions of aberrant Mxc localization in histone deficient embryos. We will then compare the genomic characteristics of novel sites to uncover similarities that might explain how Mxc is recruited to the histone genes. Additionally, by performing Mxc Dam-ID in wild type embryos, we will capture regions of the genome that contact the histone genes in three-dimensional space. Overall, our experiments will reveal new factors that contribute to transcription factor localization other than DNA sequence.

1394S **Teasing apart a bi-level neuronal function for Tip60 HAT at the chromatin and RNA level.** Christina M. Thomas,

Akanksha Bhatnagar, Felice Elefant Biology, Drexel University

The histone acetyltransferase (HAT) Tip60 is an essential epigenetic mediator of neuronal transcriptional regulation that is implicated in Alzheimer's disease (AD). Tip60 contains a catalytic HAT domain that promotes histone acetylation mediated chromatin control and a chromodomain (CD) that interacts with methylated histone lysine residues. Recently, our lab reported a novel RNA binding function for Tip60 that is localized within its CD that partakes in neuronal RNA alternative splicing (AS) regulation in the brain. Cognition and memory formation is governored by protein diversity that is possible through AS of RNA. Recent reports highlight defects in RNA splicing of genes in the brains of AD patients, thus making splicing disruptions a widespread hallmark of AD. Unfortunately, causes for these splicing disruptions in the brain are currently unknown. To further elucidate Tip60's RNA binding/splicing function, we carried out high resolution homology modeling and molecular visualization of Tip60's chromodomain (CD) that strongly predicted an RNA binding loop within Tip60's CD that's critical for direct Tip60-RNA interaction. To tease apart Tip60's RNA versus histone binding function in neural gene control and cognition, we mutated highly conserved amino acids (a.a) in Tip60's CD strongly predicted to specifically interact with either histones ($Tip60^{mutHis}$) or RNA ($Tip60^{mutRNA}$) and generated transgenic flies carrying these inducible mutant Tip60 constructs. These transgenic $Tip60^{mutRNA}$ and $Tip60^{mutHis}$ fly models will serve as powerful tools to tease apart neural functions dependent upon histone vs. RNA binding or both. We will induce expression of mutant Tip60 in the brain and carry out functional assays to assess cognitive ability using both larval and adult learning and memory assays as well assess brain morphology using immunohistochemistry. We will also assess gene expression using RNA-Seq, Tip60 splicing activity using rMATs on RNA-Seq data, and chromatin and RNA binding using ChIP and RIP, respectively. We anticipate that RNA versus histone binding functions are required for specific functional outputs and some neuronal processes will be more dependent on a given Tip60 binding function than others. Our results will elucidate a new bi-level regulatory role for Tip60 in chromatin and RNA that has potential to transform how researchers view Tip60 HAT mediated neural gene control in the context of cognition and AD.

1395S **Dissecting the functions of the RNA-binding, LCD-containing protein Rbfox1 in Drosophila ovaries** Ona Marija Singh[1,2], Halyna R Shcherbata[1,2] [1]Shcherbata Lab, Mount Desert Island Biological Laboratory, [2]Institute of Cell Biochemistry, Hannover Medical School

Drosophila Rbfox1 is a human RBFOX protein family homolog, deregulation of which is associated with various multiple human diseases, such as autism, diabetes, obesity, epilepsy and spinocerebellar ataxia. Rbfox1 is an RNA-binding protein and has been previously shown to participate in alternative RNA splicing in the nucleus; however, its role in forming liquid granules has been less studied. We have previously demonstrated that Rbfox1 is included in various different types of ribonucleoprotein (RNP) granules, such as nucleoli, Cajal bodies, stress granules and P bodies. We have also shown that Rbfox1 dysregulation leads to abnormal stress response and that its expression is regulated by a stress-related microRNA, miR-980. Furthermore, it has been demonstrated that Rbfox1 contains a highly conserved RNA recognition motif (RRM), which binds a specific UGCAUG nucleotide sequence. In addition, our data indicate that Rbfox1 has multiple low complexity sequence domains (LCDs), which are important for liquid-liquid phase separation into membrane-less organelles in the cytoplasm as well as in the nucleus. To understand the role of Rbfox1 domains, we have generated CRISPR mutant flies that lack either the RRM or one of the LCDs. We found that these mutants have different survival rates at different developmental stages. Furthermore, the observed phenotypes in CRISPR/Cas9 mutants are cell- and differentiation state-specific and also depend on the presence of stress. This demonstrates that different Rbfox1 domains and their respective roles are differentially required in different cell types in vivo. We have also generated Rbfox1 overexpression constructs to verify a cell-autonomous effect of Rbfox1 dysregulation. Overexpression of these constructs in several tissue types results in lethality, which underlines the importance of regulation to maintain proper Rbfox1 levels. A further analysis of CRISPR/Cas9 mutants and animals with tissue-selective Rbfox1 overexpression will help to dissect the functional role of Rbfox1 domains and a potential interaction through its binding partners. This knowledge will help to further our understanding of how the deregulation of Rbfox1 contributes to deleterious symptoms, which comprise a vast spectrum of human disorders.

1396S **Transvection and analysis of allele-specific expression as a pairwise contest** Andrew G Clark[1], Elissa J Cosgrove[2] [1]Molecular Biology and Genetics, Cornell Univ, [2]Molecular Biology and Genetics, Cornell University

Transvection is a genetic phenomenon whereby a heterozygote for two different defective alleles may have wild-type function, and disruption of somatic homolog pairing disrupts this rescue. While transvection has been amply demonstrated at a handful of genetic loci in Drosophila (e.g. *bicoid, yellow*), an outstanding question in genetics is whether the entire genome has this property. Here we apply deep and well replicated RNA-seq to female head tissue from progeny of a grid cross of five founder lines from the Drosophila Synthetic Reference Population, and test how often allele-specific expression levels suggest any inter-allelic interaction. The read counts can be thought of as a pairwise contest between alleles, and we fitted the Bradley-Terry model to assess goodness-of-fit to the null hypothesis of no interaction. Of the 10,139 genes detected, 8,053 had sufficient data to call allele-specific expression across the F1 hybrids and fit the Bradley-Terry model. The majority of genes

fail to reject the Bradley-Terry model (consistent with zero inter-homolog interaction), but there is an interesting subset of 147 genes that reject the model (at FDR < 0.1), sometimes in striking ways. These cases imply that there may be some form of cross-talk among alleles, possibly including transvection, that perturb allele-specific expression. Such non-independence of allelic expression could impose some interesting properties on the evolutionary fate of mutant alleles, as their marginal fitness effects would depend in a frequency-dependent manner on other alleles in the population.

1397S **Using proximity labeling with TurboID to study Polycomb Repressive Complexes in *Drosophila*.** Enya J Selders[1], Mitzi I Kuroda[2,3], Janel Cabrera[4] [1]Biology, Emmauel College, [2]Medicine, Brigham and Women's Hospital, [3]Genetics, Harvard Medical School, [4]Biology, Emmanuel College

Polycomb repressive complexes (PRCs) play an important role in regulating gene silencing during development and differentiation. These complexes establish and maintain the silent state by catalyzing repressive histone modifications. There are two major canonical complexes, PRC1 and PRC2, that have been extensively studied from *Drosophila* to mammals. Mammalian studies have also identified five variant PRC1 (vPRC1) complexes that function in gene repression. These vPRC1 complexes have been implicated in initial Polycomb group protein (PcG) targeting, a step that is currently not well understood. Recent findings from our lab have discovered that *Drosophila* has vPRC1 complexes that are homologous to the complexes seen in mammals. We propose utilizing a new method, TurboID, to analyze these subunits in early developmental time windows. The promiscuous BirA enzyme is able to biotinylate interacting proteins rapidly at a temperature suitable for *Drosophila* culture. Using this system, we tagged known proteins in *Drosophila* S2 cells in order to identify new interactors as proof of concept for earlier time frames of development. Using this approach, we study the Pc and MSL3 proteins which are subunits of chromatin modifying complexes in different pathways to determine the specificity in our system. In preliminary data, TurboID has been successful in biotinylating the proteins of interest and interactors in S2 cells. Dissecting the composition and function of vPRC1 complexes through TurboID labeling in a simple model organism like *Drosophila* will provide an important complementary approach to mammalian studies of Polycomb repressive complexes in development and disease.

1398S **The Role of the Drosophila Muscle Gene CG42319 in Muscle Development and Function** Ebru Robinson, Richard M. Cripps Biology, San Diego State University

Z-discs, fundamental elements of muscle structure, play a pivotal role in anchoring actin filaments using α-actinin molecules. The Z-disc serves as a crucial anchoring point for thin filaments during muscle contraction. Consequently, discerning the constituents of the Z-disc is important to gain a comprehensive understanding of myofibril assembly and its functional mechanisms. While the complete structure and functions of mature Z-discs remain partially understood, they are thought to require numerous proteins for assembly and maintenance. Mutations in Z-disc-related proteins have been linked to myofibrillar myopathy (MFM), a group of heterogeneous chronic neuromuscular disorders. The Drosophila gene *CG42319* is an ortholog of human PDZ LIM genes and is the focus of our study. We aim to elucidate the structural and functional role of CG42319 in Z-disc formation and function. Our research seeks to determine if the CG42319 protein is essential for the interaction between α-actinin and the Z-disc in flight muscles, as well as for maintaining normal sarcomere structure. To investigate the function of the CG42319 gene further, we generated several mutant alleles using CRISPR/Cas9 gene editing technology, aiming to uncover the gene's underlying mechanisms. Our initial findings reveal that these mutant alleles markedly disrupt α-actinin structure, causing muscle dysfunction in the indirect flight muscles of Drosophila. Importantly, mutations in human orthologs (PLIM2) of this gene have been associated with severe muscle dysfunction and structural abnormalities observed in myopathies and muscular dystrophies. Our research holds the potential to advance our understanding of the PDZ domain protein CG42319 and its significance in the field of muscle biology.

1399S **Genome-wide screening of miRNA's involved in birth defects in eye** Manivannan Subramanian[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,5] [1]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [4]Integrative Science and Engineering (ISE), University of Dayton, [5]Center for Genomic Advocacy (TCGA), Indiana State University

Aniridia, a birth defect in eye, is caused by mutation(s) in paired-box gene 6 (PAX-6), and is manifested as visual impairment. A highly conserved PAX-6, encodes a DNA-*binding* transcription factor, whose loss-of-function exhibits loss-of-entire-eye or part-of-eye. Surprisingly, the understanding of the molecular genetic basis of Aniridia is far from complete. Our hypothesis is that the retinal loss observed in aniridia can be due to post transcriptional regulation such dysregulation of miRNAs that plays a pivotal role in regulating genes post transcriptionally. miRNAs are the short hairpin like structure with 20-25bp which modulates the gene expressions post-transcriptionally by binding to 3'UTR of mRNAs. miRNA serves a vital role in the retina throughout development and in eye diseases. We employed *Drosophila eye* as a model system for genome-wide screening of miRNAs involved in eye defects. We have identified a *miRNA* which exhibits strong eye enlargement phenotype. Using bioinformatic approaches, followed by validation using molecular and genetic studies, we identified PAX-6 homolog *eyeless*

(ey) as target of this mi-RNA. Here, we provide a mechanism of how this newly identified *miRNA* modulates eye phenotype, and results from these studies will be presented.

1400S **Fat cadherin cleavage releases a transcriptionally active nuclear fragment to regulate target gene expression** Jannette Rusch[1], Chikin Kuok[2], Nattapon Thanintorn[1], Yonit Tsatskis[2], Helen McNeill[1] [1]Developmental Biology, Washington University St Louis School of Medicine, [2]Lunenfeld-Tanenbaum Research Institute, Mt Sinai Hospital

The conserved atypical cadherin *fat* (*ft*) controls cellular processes such as growth via the Hippo pathway, planar cell polarity, and mitochondrial function, in organisms ranging from fruit flies to mammals. The intracellular domain of the Ft protein, FtICD, binds to and regulates a variety of partners to execute these functions. We have found that FtICD is present in the nucleus in tissue culture cells as well as in embryonic and larval tissues, and have identified nuclear localization and nuclear export signals in FtICD required for this localization. A membrane-bound version of FtICD, fused to a Gal4VP16 transactivation domain, is able to activate a reporter construct in imaginal discs, demonstrating that FtICD can be cleaved and enter the nucleus *in vivo*. To determine if Ft regulates transcription we used CRISPR to endogenously label Ft, and conducted ChIP-Seq. Ft target genes identified through ChIP experiments on *Drosophila* embryonic and larval tissues include genes involved in signaling pathways including the Hippo pathway, chromatin organization, pattern formation, neural development and others. A subset of these genes are differentially regulated in *ft* mutants and/or animals overexpressing FtICD, as determined by RNAseq experiments. The anti-apoptotic gene, *Diap1*, a target of the Hippo pathway, is upregulated in *ft* mutants, and *in vivo* analysis of a fragment of the *Diap1* enhancer, HRE (Hippo Response Element), indicates that FtICD can activate HRE, further supporting a nuclear function for *ft*. We propose that the modulation of Hippo pathway activity constitutes one of the nuclear functions of Ft, complementing its established function as an upstream regulator of Hippo signaling.

1401S **Head-specific gene dynamics during embryonic development** Priyanshi H Borad[1], Kelli Fenelon[1], Theodora Koromila[1], Parisa Boodaghimalidarreh[2], Jacob Luber[3] [1]Biology, University of Texas at Arlington, [2]Computer Science, University of Texas at Artlington, [3]Computer Science, University of Texas at Arlington

Genomic regulation in cell development is primarily based on noncoding regulatory sequences called enhancers, in coordination with promoter activities. Chromatin accessibility is essential for interaction of transcription factors (TFs) with accessible regulatory elements. Enhancers are available to bind with the proteins that regulate gene expression once chromatin is accessible. Although the biological importance of enhancers has been previously described, it remains unclear how head-expressed transcription factors (TFs) control gene expression. In a previous study, we found that during MBT pioneer transcription factor *Opa* and Drosophila's homolog of the *Otx* family known to regulate neural development in mice, Ocelliless *(Oc/Otd)*, expression domains overlap with both factors being simultaneously transcribed within the pre-gastrula cells in the overlapping region. Here, we provide insights into *Opa*'s and *Oc*'s mechanisms of action in head-specific gene regulation by preparing *Opa/Oc* knockdown sc-RNA-seq and scATAC-seq libraries to understand how these temporal TFs regulate expression of *kdm5 (*Lysine-specific demethylase 5)/*lid* (Little imaginal discs), *hb* (hunchback), *dll* (Distal-less) genes via the 5'*lid*, *hb*_VT38545, and *Dll*_VT22064 enhancers in the *D. melanogaster* embryo. Predicted binding sites for both *Opa* and *Oc* factors were identified in the aforementioned enhancers and upon mutagenesis the gene expression patterns exhibit dynamic changes in the width of reporter-driven expression outputs. To assess these phenotypes systematically, we devised a live imaging quantitative approach to measure the spatiotemporal outputs across the entire embryo. In conclusion, our results demonstrate that *Opa* and *Oc* play temporally-distinct roles and contribute to dynamic gene expression in the developing embryonic head.

1402S **Analyzing the role of BCOR and RSF1 in variant Polycomb Repressive Complex 1.1 in *Drosophila*.** Cynthia E Brito[1], Camila A Moreno-Bo[1], Mitzi I Kuroda[2,3], Janel Cabrera[1] [1]Biology, Emmanuel College, [2]Medicine, Brigham and Women's Hospital, [3]Genetics, Harvard Medical School

Polycomb repressive complexes (PRCs) play an important role in regulating gene silencing during development and differentiation. These complexes establish and maintain the silent state by catalyzing repressive histone modifications, histone H3 lysine 27 methylation (H3K27me) and histone H2A lysine 118 ubiquitylation (H2AK118ub). There are two major canonical complexes, PRC1 and PRC2, that have been extensively studied from *Drosophila* to mammals. Mammalian studies have identified several variant PRC1 (vPRC1) complexes that function in gene repression. Until recently, it was believed that *Drosophila* did not possess similar vPRC1 complexes. However, recent findings from our lab have discovered that indeed *Drosophila* has vPRC1 complexes that are homologous to the complexes seen in mammals. This study focuses on understanding the role of vPRC1.1. We have identified two novel subunits, Remodeling and Spacing Factor 1 (RSF1) and BCL6 Corepressor (BCOR) as subunits of vPRC1.1. Previous studies in *Drosophila* and mammals have found that RSF1 (CG8677) regulates repressive chromatin assembly by reading the PRC histone modification (H2AK118ub) and through incorporation of the variant histone H2Av. Mammalian BCOR has been implicated in many human cancers including acute myeloid leukemia

and is essential for PRC1.1 function and stem cell pluripotency. In *Drosophila*, BCOR (CG14073) is a novel gene that has not been characterized. To test whether RSF1 and BCOR are Polycomb group proteins (PcG), we are conducting genetic crosses between mutant Polycomb, RSF1, and BCOR lines to analyze homeotic transformations of the sex combs of male *Drosophila*. We will also analyze the role of these proteins during development by generating null mutants and analyzing the effects on embryonic development. Dissecting the function of the vPRC1.1 complex by studying RSF1 and BCOR in a simple model organism like *Drosophila* will provide an important complementary approach to mammalian studies of Polycomb repressive complexes in development and disease.

1404S **Molecular mechanisms underlying neural-specific splicing and 3'UTRs** Xin Yu Zhu Jiang[1,2], Lu Wei[3], Seungjae Lee[3], Eric C. Lai[3] [1]BCMB, Weill Cornell Graduate School, [2]Development Biology, Memorial Sloan Kettering Cancer Center, [3]Developmental Biology, Memorial Sloan Kettering Cancer Center

The conserved family of ELAV/Hu RNA binding proteins (RBPs) regulates mRNA processing in neurons. They expand transcriptional diversity by inducing alternative splicing (AS) of internal exons and alternative polyadenylation (APA) to lengthen 3'UTRs. Ectopic assays show that ELAV/Hu RBPs are sufficient to confer neural-like splicing and 3'UTR isoforms in a non-neural setting, and are correlated with direct binding at targets. However, mechanistic insights for how ELAV/Hu RBPs direct all of these neural isoform programs is elusive. To understand this, we propose to conduct immuno-precipitation and proteomics for ELAV/Hu partners, which may include the spliceosome complex and/or cleavage and polyadenylation (CPA) factors. Furthermore, building on data that Elav binds to U-rich regions in regions flanking alternative neural exons, or at alternative pA sites, we will map specific binding sites and characterize their regulatory grammar that can direct alternative neural isoform programs.

1405S **Investigating age-associated histone modification patterns as a regulator of lifespan** Devonique L Brissett[1], Jeff Leips[1], Erin Green[2] [1]Biological Sciences, University of Maryland Baltimore County, [2]University of Maryland Baltimore County

Aging is accompanied by dynamic changes in the levels of histone modifications. While the prevalence of histone modifications can directly regulate lifespan, this effect is often tissue-specific. Substantial variation in lifespan exists both across and within several species. Genetic differences between *Drosophila melanogaster*lines have been implicated in lifespan variation. Previous studies using young and old flies revealed that expression of a putative *Drosophila* histone methyltransferase, *SmydA-5,* was shown to increase with age in a short-lived genotype of the *Drosophila* Genetic Reference Panel (DGRP) 73, while histone deacetylase *Sirt2*expression was shown to increase in a long-lived genotype, DGRP 304, during aging. Whether these differences in histone modification abundance contribute to differences in lifespan between the genotypes is currently unknown. In this study, we are using mated males and females from three genotypes with differing lifespans to quantify the abundance of H3K4me3 and H4K16ac in the fat body tissue and thorax at three ages. Preliminary findings have revealed that global levels of H3K4me3 increase during aging in the fat body tissue in both males and females. We observed that females from the longest-lived line had stable levels of H3K4me3 with age, while the two shorter-lived lines had modest increases of H3K4me3. Upon analyzing global levels of H4K16ac with age, we found that levels increased during aging in the fat body tissue in males and females. Among males, the shortest-lived line had the highest increase of H4K16ac, while the two longer-lived lines showed lower increases of the mark. Current studies are underway to determine patterns of these marks in the thorax during aging. This work provides the basis for understanding tissue-specific histone modification changes during aging and determining how epigenetic regulation contributes to lifespan variation.

1406S **Understanding the gene regulation dynamics in embryonic heart development** Shiva Abbasi[1], Lisa Phan[1], George Tegousis[2], Theodora Koromila[1] [1]Biology, The University of Texas at Arlington, [2]Biology, Aristotle university of Thessaloniki

Embryonic patterning systems direct a set of initially uncommitted pluripotent cells to differentiate into a variety of cell types and tissues. Cell-type specification and differentiation occur early in development, and the core network of Transcription Factors (TFs) that lead to organogenesis are conserved in evolution. The *Drosophila* embryo is adapted for speedy development, as the early embryo is composed of a syncytium lacking cellular compartments until fourteen cycles of synchronized nuclear divisions have occurred. Thus, *Drosophila* is a powerful system to study genes involved in heart formation and the mechanisms of heart failure. Recent studies have shown during cellularization zygotic activation is regulated by pioneer factors such as, Odd-paired (Opa) and GAGA factor (Gaf) and other transcription factors, such as Twist (Twi) and Runt. Our hypothesis is Opa, Twi, Gaf are present in the cellularized embryo at the same time and regulating cardiac genes, such as early and late enhancers in *sloppy paired* (*slp*), *paired* (*prd*), and *tailup* (*tup*) genes. Opa have long been known to be involved in heart development and mutations in this TF produce heart defects in humans, mice, and flies, however its role in the early embryo is still unclear. Combining fluorescent In situ hybridization, live-imaging microscopy, and multi-omics, we provide insights on how cellularization-expressed transcription factors regulate heart cell specification in the early embryo.

1407S **A polycistronic non-coding RNA locus regulates germline differentiation and testis morphology in Drosophila** Travis

D Carney[1,2], Halyna R. Shcherbata[1,2] [1]Mount Desert Island Biological Laboratory, [2]Institute of Cellular Biochemistry, Hannover Medical School

Using the Drosophila testis as a model to investigate novel functions of non-coding RNAs, we found that the microRNA *miR-317* plays a role in the differentiation of germline stem cell progeny. *miR-317* and its neighbor, the lncRNA *Peony,* originate and are co-expressed from a singular polycistronic non-coding RNA locus, with alternative polyadenylation implicated in the regulation of their differential expression. While the increased expression of *Peony* causes a disruption of the muscle sheath covering the testis and leads to a dramatic enlargement of the testis apex, the absence of *miR-317* results in the emergence of germline tumors in young flies. These tumors arise near the testis apex during development, migrate basally in adult flies, and finally degrade near the terminal epithelium region of aging testes. The deficiency of *miR-317* increases Notch signaling activity in somatic cyst cells, which promotes germline tumorigenesis, while reducing Notch signaling strength decreases the incidence of tumors. Germline tumors also result from somatic upregulation of several predicted targets of *miR-317*, among which are regulators of the Notch pathway. This work demonstrates that *Peony* is a functional lncRNA regulating testis structural integrity and implicates *miR-317* as a novel tumor suppressor that simultaneously represses multiple target genes and modulates Notch signaling strength in that role.

1408S **Nucleotide-level distance metrics to quantify alternative splicing implemented in *TranD*** Adalena Nanni[1], James Titus_McQuillan[2], Kinfeosioluwa Bankole[1], Alison Morse[1], Rebekah Rogers[2], Ana Conesa[1], Lauren McIntyre[1] [1]University of Florida, [2]University of North Carolina

Advances in affordable transcriptome sequencing combined with better exon and gene prediction has motivated many to compare transcription across the tree of life. In this work, we develop a mathematical framework that uses distance metrics to calculate complexity and compare transcript models from a structural perspective. Structural features, i.e., intron retention (IR), donor/acceptor site variation, alternative exon cassettes, alternative 5'/3' UTRs, are compared and the distance between transcript models is calculated with nucleotide level precision. All metrics are implemented in a PyPi package, *TranD*, that can be used to summarize splicing patterns for a transcriptome (1GTF) and between transcriptomes (2GTF). *TranD* enables quantitative comparisons between: annotations augmented by empirical RNA-seq data and the original transcript models; transcript model prediction tools for longread RNA-seq (e.g., FLAIR vs Isoseq3); alternate annotations for a species (e.g., RefSeq vs Ensembl); and between closely related species. In both annotation and long read RNA-seq for *C. elegans, Z. mays, D. melanogaster, D. simulans* and *H. sapiens*, alternative exons were observed more frequently in combination with an alternative donor/acceptor than without. Data from *D.melanogaster* and *D.simulans* suggest that both species are under-annotated and single transcript genes are likely to share the same transcript model.

1409S **The roles of lncRNAs in the expression and processing of Y chromosome mega-genes** Matthew Jachimowicz[1,2,3], Lingfeng Ma[1,2,3], Zhantao Shao[2], Jack Hu[2], Chun Hu[2], Julie A. Brill[1,3], Henry M. Krause[1,2] [1]Molecular Genetics, University of Toronto, [2]Donnelly Centre, University of Toronto, [3]Cell Biology Program, The Hospital for Sick Children

In *Drosophila* and mammals, long non-coding RNA (lncRNA) abundance and diversity is highest in male reproductive tissues, yet their potential roles in male reproduction are largely unexplored. To address this nascent field, I have begun characterizing a group of testis-expressed lncRNAs, that we have shown to spatially associate with megabase-long genes or 'mega-genes'. These novel nuclear associations suggest that lncRNAs may regulate mega-genes at a transcriptional or post-transcriptional level. Additionally, these lncRNAs may directly interact with mega-genes by complementary hybridization, or indirectly by hybridizing to an intermediate lncRNA. To test my hypothesis, I have computationally identified complementary sequences between mega-gene associated lncRNAs and simultaneously compared their expression patterns using fluorescent in situ hybridization (FISH). Indeed, mega-gene associated lncRNAs sharing complementary sequence colocalize, maintaining the possibility of an interaction. To further investigate these associated lncRNAs, I will test whether this is a functional association between mega-genes and lncRNAs using a CRISPR knockout approach. Furthermore, lncRNA functions will be further characterized by identifying their interactors and interacting sequences using a hybridization-dependent biotinylation method. Ultimately, my results will potentially contribute to our understanding of mega-gene regulation in human contexts, such as in the regulation of *dystrophin* and human Y-linked mega-genes, and begin addressing the roles of lncRNAs in male reproduction.

1410S **Study of piRNA clusters, loci involved in genome stability** Zoheir Ziriat, Teysset Laure, Carré Clément UMR7622, Sorbonne Université

piRNA clusters are heterochromatic loci involved in the maintenance of genome integrity by ensuring the control of transposable elements (TE) *via* the production of small noncoding RNAs called piwi-interacting RNAs (piRNAs). Despite the important role of these loci in genome stability, we know really few things about their transcription and regulations. In our lab we're studying 2 models of germinal piRNA clusters of *Drosophila melanogaster.* The sub-telomeric 1A locus, which

contains sequences unique amongst piRNA clusters that could be used to mimic the cooptation of a new transposon in *Drosophila* genome. In the other hand, BX2 is an artificial piRNA cluster that exists in 2 different epigenetic states: either active for the production of piRNAs (ON), or inactive for piRNA production (OFF) while still being transcribed. Studying these 2 loci using functional tests, molecular tools and 3rd generation sequencing approaches will allow us to decipher the complex biology of piRNA clusters

1411S **Pilot screen to identify genes that participate with H4K20 methylation in controlling cell proliferation in *Drosophila*** Kelli Jancay[1], Marybeth Slack[1], Aaron Crain[2], Robert J Duronio[2,3,4,5], Lisa M Antoszewski[1] [1]Biology, Grove City College, [2]Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, [3]Biology, University of North Carolina at Chapel Hill, [4]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, [5]Genetics, University of North Carolina at Chapel Hill

Control of cell proliferation is essential for organismal development and survival and occurs through a series of steps called the cell cycle. During the cell cycle, the genome is copied before the cell divides and, if this process is compromised, DNA damage and death can result. The ability to effectively replicate DNA and therefore control cell proliferation is affected by chromatin structure and accessibility, which is influenced by post-translational modifications to histone proteins including methylation. Data suggest that cell proliferation is compromised when H4K20 methylation is disrupted. However, the exact mechanism behind a requirement for H4K20 methylation in the cell cycle is still unknown. Our goal is to identify genes on the third chromosome of *Drosophila* that regulate cell proliferation through H4K20 methylation. Using a histone deletion on the second chromosome and a transgene on the third chromosome that expresses either wild type or H4K20R mutant histone, we will utilize the FLP/FRT system to create histone mutant clones in the adult eye. Wildtype cells will be marked with GFP and histone mutant cells with RFP, and the size of histone mutant clones relative to wildtype clones will be determined with fluorescence microscopy. To identify genes that interact with H4K20 methylation, we plan to perform a pilot screen with Set8-interacting proteins. Set8 is the only known H4K20 histone mono-methyltransferase in eukaryotes, and the action of Set8 is required for subsequent di and tri methylation of H4K20. The size of histone mutant clones will be analyzed when heterozygous loss of function mutations in genes encoding Set8-interacting proteins are introduced. Following the completion of this pilot screen, a forward genetic screen using the 3rd chromosome deficiency kit will be executed.

1412S **Investigating the *in vivo* functions of Histone 3 (H3) monoaminylation using *Drosophila*** Harim Delgado-Seo[1], Gary Huang[2], Herman A. Dierick[2], Ian S. Maze[3], Shinya Yamamoto[4] [1]Neuroscience, Baylor College of Medicine, [2]Molecular and Human Genetics, Baylor College of Medicine, [3]Departments of Neuroscience and Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, [4]Department of Molecular and Human Genetics, Baylor College of Medicine

Breakthroughs in epigenetic discoveries have revealed epigenetic mechanisms that can induce lasting changes in both the structure and function of the nervous system, thereby influencing a spectrum of neurological and psychiatric disorders. Recent work has found that biogenic monoamines like dopamine and serotonin can post-translationally modify Histone 3 (H3) at two specific glutamine (Q) residues, H3-Q5 and H3-Q19. Initial studies of this process, known as H3 monoaminylation, found that transcriptional and behavioral patterns of cocaine-dependent rats were partially reversible by overexpressing a monoaminyl-deficient H3-Q5 variant in the reward circuit. Although H3 monoaminylation has been henceforth linked to mood disorders and other drug addictions, its overall physiological importance and the functional significance of the second monoaminyl site (H3-Q19) remains unclear. Therefore, this study aims to probe the functional significance of both H3 monoaminylation sites *in vivo* by using throughput genetic and behavioral techniques in the fruit fly. To investigate the effects of monoaminylation on H3-Q5 and H3-Q19, *Drosophila* strains with H3 variants mimicking unmodifiable glutamine (Q) residues were designed. This was achieved by amino acid substitutions replacing glutamine (Q) residues with alanine (Q>A) or asparagine (Q>N), in the individual (Q5 or Q19) or combined (Q5+Q19) monoaminylation sites using phiC31-mediated targeted integration. Thus far, we have found that ubiquitous overexpression of individual H3-Q>A and H3-Q>N variants resulted in comparable semi-lethality patterns, suggesting that monoaminyl deficiency rather than specific amino acid substitutions is responsible for this phenotype. Moreover, pan-neuronal overexpression of H3-Q>A variants causes defects in wing expansion behavior, which is regulated by a genetically and neurobiologically traceable neuro-hormone signaling cascade. Therefore, we have found a potential pathway for further exploration into the precise function of this novel epigenetic mark *in vivo*.

1413S **Nutrient-dependent regulation of the *Drosophila melanogaster* Estrogen Related-Receptor (ERR)** Sophie A Fleck, Hongde M Li, Maria C Sterrett, Jason M Tennessen Department of Biology, Indiana University Bloomington

Animal development is acutely sensitive to environmental conditions including dietary nutrients, temperature stress, and xenobiotic compounds. One of the key sensors involved in coordinating environmental cues with developmental events are the highly conserved family of nuclear receptor transcription factors. In this regard, the Estrogen-Related Receptor (ERR) family of orphan nuclear receptors play a key role in coordinating cellular metabolism with developmental gene expression programs.

We previously demonstrated that the *Drosophila melanogaster* ortholog of ERR activates a metabolic program known as aerobic glycolysis prior to the onset of larval development, thus establishing a metabolic state that supports rapid juvenile growth. During this embryonic switch, which occurs approximately 12 hours before hatching, accumulation and activation of ERR protein directly activates transcription of genes encoding enzymes within glycolysis and the pentose phosphate pathway (PPP). Intriguingly, we've found that expression of ERR protein earlier in embryonic development does not drive precocious activation of aerobic glycolysis. However, premature expression of a constitutively active ERR-VP16 fusion protein is capable of inappropriately activating target gene expression. Together, these findings indicate that ERR activity is controlled at a post-translational level via an unknown mechanism. Consistent with these embryonic studies, we've also demonstrated that ERR activity during larval development tightly correlates with nutrient availability, as ERR become inactive when *Drosophila* larvae are fed a nutrient poor diet. Further analysis by western blot revealed an approximate 40 kDa shift in the weight of ERR when larvae are starved. These findings indicate that ERR may be regulated through inhibitory post-translational modification(s) (PTMs) when environmental conditions are inadequate for *Drosophila* development. Considering that ERR family members are orphan receptors that are highly conserved across evolutionary time, our findings are important because they hint at an unknown mechanism that controls post-translational ERR activity. Finally, our studies indicate that ERR coordinates developmental metabolism with nutrient availability.

1414S **Layered regulation of the Drosophila CTLH complex during the maternal-to-zygotic transition controls maternally-deposited RNA binding protein clearance** Chloe Briney[1], Jesslyn Henriksen[2], Olivia Rissland[1] [1]University of Colorado Anschutz Medical Campus, [2]University of Colorado, Anschutz Medical Campus

The maternal-to-zygotic transition (MZT) is a conserved and essential early developmental process that ensures embryogenesis occurs properly after fertilization. Importantly, prior to zygotic genome activation, the early Drosophila embryo proteome consists mainly of maternally deposited proteins. During the MZT, maternal gene products are cleared to make way for newly transcribed and translated zygotic gene products. A key step of the MZT is therefore the removal of specific maternal proteins. One well-characterized example is the clearance of the maternally deposited ME31B-Cup-TRAL RNA binding protein complex, which we recently found to be cleared by the translationally-upregulated E2 Kondo and CTLH E3 complex (Zavortink et al 2020). However, ME31B and many components of the CTLH complex are ubiquitously expressed in flies, so why and how is ME31B clearance so tightly regulated during the MZT? Our findings reveal a complex gene regulatory network that mediates the developmental specificity of ME31B degradation. This network relies on the modularity of the ubiquitin proteasome system and its ability to exert precise control by restricting expression of its components to specific timeframes. This network involves at least four layers of control. First, E2 Marie Kondo mRNA is only translated at the start of the MZT. Second, the CTLH subunit Muskelin is required for substrate recognition and is only expressed during oogenesis. Third, recognition of ME31B by the CTLH complex and its interaction with Muskelin changes during the MZT. Finally, Marie Kondo and some CTLH components, including Muskelin, are themselves degraded at the end of the MZT. Our results provide evidence for an elegant network of regulatory mechanisms controlling degradation of specific deposited maternal proteins with precise developmental timing.

1415S **BRD4 binds to active cranial neural crest enhancers to regulate RUNX2 activity during osteoblast differentiation** Trevor V. Shumate, Karl Shpargel Department of Genetics, University of North Carolina

Cornelia de Lange syndrome (CdLS) is a congenital disorder featuring facial dysmorphism, postnatal growth deficits, cognitive disability, and upper limb abnormalities. CdLS is genetically heterogeneous with cases arising from mutation of BRD4, a bromodomain protein that binds and reads acetylated histones. In this study we have modeled CdLS facial pathology through mouse neural crest cell (NCC) specific mutation of BRD4 to characterize cellular and molecular function in craniofacial development. Mice with BRD4 NCC loss of function died at birth with severe facial hypoplasia, cleft palate, mid-facial clefting, and exencephaly. Following migration, BRD4 mutant NCCs initiated RUNX2 expression for differentiation to osteoblast lineages but failed to induce downstream RUNX2 targets required for lineage commitment. BRD4 bound to active enhancers to regulate expression of osteogenic transcription factors and extracellular matrix components integral for bone formation. RUNX2 physically interacts with a carboxy-terminal (C-terminal) domain in the long isoform of BRD4, can co-occupy osteogenic enhancers, and this association is required for RUNX2 recruitment and appropriate osteoblast differentiation. We conclude that BRD4 controls facial bone development through osteoblast enhancer regulation of the RUNX2 transcriptional program.

1416S **Interrogating the roles of KMT2C and KMT2D in chondrocyte differentiation and endochondral ossification** Gabrielle A Quickstad[1], Dimitri V Bikas[2], Karl B Shpargel[1] [1]Genetics, UNC Chapel Hill, [2]UNC Chapel Hill

Chromatin-modifying enzymes associated with enhancer function are frequently mutated in human craniofacial disorders. Kabuki syndrome (KS) is a craniofacial development disorder characterized by mutations in KMT2D, a histone H3 lysine 4 (H3K4) methyltransferase. Neural crest cells (NCCs) are the stem cells from which anterior facial bone and cartilage originate. Our preliminary studies show that mouse NCC deletion of KMT2D results in a lack of cranial base chondrocyte hypertrophic

differentiation. Many craniofacial disorders linked to mutations in enhancer-associated chromatin-modifying enzymes, including KS, present with a wide range of skeletal abnormalities in humans including shortened long bones, scoliosis, and joint abnormalities. As these patients present with skeletal defects that may result from abnormal chondrocyte differentiation, understanding the mechanism of KMT2D function in this differentiation process is crucial to a better understanding of both craniofacial disorders and skeletal development. A conditional loss of KMT2D and its functional homolog KMT2C (KMT2C/D DKO) in mouse chondrocytes yields a dramatic reduction in weight at weaning, shorter fore and hindlimb bone lengths, and a decrease in size of the presphenoid and basisphenoid regions of the cranial base. Safranin O-fast green FCF staining of cartilage in mouse hindlimbs reveals a loss of columnar epiphyseal growth plate chondrocyte organization, thickening of epiphyseal growth plates of the tibia and femur, and overgrowth of articular cartilage into the intercondylar fossa. Subsequent high-resolution imaging will shed light on bone composition, type, and density alterations in KMT2C/D DKO mice as compared to wildtype. We are currently exploring KMT2D's molecular mechanism by interrogation of KMT2C/D-deficient NCCs and primary mesenchymal stem cells in culture for changes in KMT2D genome binding, chromatin accessibility, differential gene expression, and localization of enhancer histone-methylation during chondrocyte differentiation. We will ascertain the novel functional roles that KMT2C and KMT2D play in regulating chondrocyte differentiation and the results of this study will implicate epigenetic modifiers in the progression and treatment of skeletal development disease.

1417S **Mechanisms of acute exercise on brain health** Tiffany Chin, Hume Akahori Stroud Neuroscience, UT Southwestern Medical Center

Exercise has long been stated as beneficial for neural health by reducing anxiety, promoting neuroplasticity, and preventing neurodegeneration. Long-term exercise mouse models have been shown to recapitulate these aforementioned benefits. However, the specific pathways involved in promoting these benefits in brain and behavior have not been clearly defined. To address this, I utilized a short-term exercise mouse model to study the immediate molecular and cellular effects of exercise on brain health. Strikingly, I found that a single bout of exercise is sufficient to stimulate neural activity of many cells in the hippocampus, a brain region critical for learning and memory. As transcriptional changes may play a role in promoting brain health in response to exercise, I aim to reveal how transcription is modulated across different cell types of the hippocampus. RNA sequencing and assay for transposase-accessible chromatin (ATAC) sequencing will be performed to observe gene expression and chromatin accessibility changes at single cell resolution, respectively. By studying transcriptional variations and chromatin conformation changes, I expect to uncover how downstream pathways are regulated and mediate the benefits of exercise.

1418S **Receptor choice in the olfactory system of ants** Bogdan Sieriebriennikov[1], Olena Kolumba[1], Aurore de Beaurepaire de Louvagny[1], Hua Yan[2], Claude Desplan[1] [1]New York University, [2]University of Florida

In both flies and vertebrates, smell is perceived by olfactory sensory neurons (OSNs) that rely on the expression of a single odorant receptor (*Or*) gene per cell to discriminate between odors. Flies have few *Or* genes (~60), while mammals have many (>1,000 in mice), which is reflected in different specification mechanisms of developing OSNs in these species. The simple olfactory system of flies uses deterministic specification of cell fates by a transcription factor code, while the complex olfactory system of mammals relies on stochastic choices, including stochastic enhancer-promoter interactions, complemented by negative cross-regulation between *Or*s. Ants, which extensively rely on smell to navigate their physical and social milieu, lie in the middle of this spectrum. The jumping ant *Harpegnathos saltator* has an order of magnitude more *Or* genes than *Drosophila melanogaster* (~400). The additional genes have evolved via tandem duplications, creating large genomic arrays of *Or* genes positioned head-to-tail, an arrangement reminiscent of *Or* arrays in mammals. We investigated the expression pattern of *Or*s in individual OSNs in *H. saltator* by performing single-nucleus sequencing on the antennae. Unexpectedly, we observed that all genes in tandem *Or* arrays are co-expressed in a "stair step" fashion where either the most 3' gene is expressed alone, or the two most downstream genes are co-expressed, or the last three genes are co-expressed etc. Additionally, the expression levels of multiple co-expressed genes show a decreasing trend towards the most 3' transcribed *Or*, suggesting that such co-expression may result from RNA polymerase readthrough. We propose that a single *Or* promoter is stochastically chosen from a cluster, activating the transcription of the corresponding gene. However, RNA polymerase fails to terminate at the end of this gene, producing long RNAs. While they include all the downstream *Or* sequences, those cannot be translated due to their distance from the 5' cap. At the same time, upstream *Or* genes are repressed through additional mechanisms which we are currently investigating. Thus, genomic clusters of ant *Or*s, which have evolved to accommodate an expanded repertoire of odors related to the ants' lifestyle, may rely on a unique mechanism to produce singular OR protein expression in each OSN.

1419S **Transcriptomic analysis reveals regulation of adipogenesis via long non-coding RNA, alternative splicing, and alternative polyadenylation** Salwa Mohd Mostafa[1], Luyang Wang[2], Bin Tian[2], Joel Graber[3], Claire Moore[1] [1]Tufts University, [2]The Wistar Institute, [3]MDI Biological Laboratory

Obesity is characterized by dysregulated adipogenesis leading to increased number and/or size of adipocytes. Understanding the molecular mechanisms governing regulation of adipogenesis is therefore key to designing therapeutic interventions against obesity. In our study, we analyzed 3'-end sequencing data generated from sequencing of human preadipocytes and adipocytes, as well as data from publicly available databases, to propose mechanisms of molecular regulation of adipogenesis. We discovered lncRNAs that have not been previously characterized but may be key regulators of both brown and white adipogenesis. We also demonstrated possible mechanisms of direct or indirect regulation of adipogenesis through alternative splicing. Finally, we show that usage of alternative polyadenylation sites of key adipogenesis genes leads to isoform diversity, which can have significant biological consequences on differentiation efficiency. Therefore, our research reveals potential therapeutic avenues for obesity through manipulation of long non-coding RNA levels, alternative splicing and usage of alternative polyadenylation sites.

1420S **Probing nuclear HSATII RNA biomolecular condensates** Thembalami Dube[1,2,3], Christina A Rabeler[1], Dawn M Carone[1] [1]Biology, Swarthmore, [2]Biology, Swarthmore College, [3]Physics, Swarthmore College

Human satellite sequence II (HSATII) is a tandemly repeated pericentric and heterochromatic sequence that is transcriptionally silent in normal human cells, yet in cancer cells HSATII RNA is aberrantly expressed and accumulates large nuclear foci that remain in cis at their sites of transcription. Expression and aggregation of HSATII RNA is concomitant with sequestration of key chromatin regulatory proteins known to phase separate including MeCP2. While HSATII expression is highly correlated with higher grade tumors in various epithelial cancer cell lines, the effect of its nuclear misregulation is poorly understood. Due to the upregulation of HSATII RNA expression in cancer, accumulation of the HSATII RNA in cis, sequestration of key regulatory phase-separating proteins, and its repetitive nature, we hypothesized that HSATII RNA is a molecular scaffold that nucleates and forms phase-separated biomolecular condensates in vivo. Condensates are increasingly recognized as important for cellular function and dysfunction. Here, we present evidence that HSATII RNA assembles into biomolecular condensates via in vitro assays. We also employ an in vivo disruption assay that utilizes doxorubicin and 1,6-hexanediol, both drugs known to specifically target and disrupt condensates in vivo. Treatment with both these drugs resulted in significant redistribution of HSATII RNA in living cells. Characterizing HSATII RNA focal accumulations as condensates provides a mechanistic account for pathological phenotypes in cancer. While this study serves to demonstrate that HSATII RNA foci can form condensates, it also has broader implications for drug development in cancer therapeutics.

1421S **Search for proteins with roles in promoting yFACT dissociation from 3' ends of transcribed genes** Will Griffin, Reece Forrest, Michaela Edwards, Joseph Beard, Lillian Francis, Agustin Kalinowski, Sydney Ozersky, Caroline Tackett, Andrea Duina Department of Biology, Hendrix College

Eukaryotic cells use histone chaperones to manipulate chromatin for a variety of processes. FACT (Facilitates Chromatin Transactions) is a highly conserved histone chaperone complex, composed of Spt16 and Pob3 in *S. cerevisiae*, that during transcription elongation has roles in the disassembly of nucleosomes in front of RNA polymerase II (Pol II) and in nucleosome reassembly in the wake of Pol II passage. Our previous research identified a region on nucleosomes known as ISGI (Influences Spt16-Gene Interactions), whose integrity is necessary for proper dissociation of yeast FACT (yFACT) from the 3' ends of transcribed genes. In addition to causing yFACT dissociation defects, the more severe ISGI mutants confer a variety of growth phenotypes, including slow growth, cold sensitivity, and in some cases lethality. Current studies in the lab aim at identifying proteins with possible roles in promoting yFACT dissociation from genes. Using a synthetic gene array strategy with cells expressing an ISGI mutant as the query strain, we identified ~150 proteins as candidates for contributing to yFACT dissociation from genes. We are currently validating these genetic interactions and are using chromatin immunoprecipitation (ChIP) assays to test the most promising candidates for possible roles in promoting yFACT-gene dissociation. We expect that these experiments will lead to the identification of one or more factors that promote yFACT dissociation from chromatin, which would in turn provide new insights into yFACT-chromatin dynamics.

1422S **RNA polymerase II-general transcription factor interfaces function in transcription start site selection in *Saccharomyces cerevisiae*** pratik basnet[1], Yunye Zhu[2], Irina O. Vvedenskaya[3], Payal Arora[2], Staci Hammer[4], Shawn Alex[5], Brittany McVicar[2], Bryce E. Nickels[6], Craig D. Kaplan[2] [1]Bio-Science, University of Pittsburgh, [2]University of Pittsburgh, [3]Rutgers University, [4]Loras College, [5]Texas A&M, [6]Waksman Institute, Rutgers University

During transcription initiation in *Saccharomyces cerevisiae*, Pol II and its general transcription factors (GTFs) assemble upstream of transcription start sites (TSSs) to form the pre-initiation complex (PIC). The PIC then selects TSSs by a unidirectional scanning mechanism. Structural studies show that a TFIIH component Tfb3 links TFIIH to the rest of the PIC via its interactions with Pol II and TFIIE. Activities within the PIC that influence TSS selection can do so by control of initiation efficiency at individual TSSs and by control of TSS scanning (either rate of scanning or scanning processivity). How factors in the PIC at the interface of Pol II and TFIIH contribute to these different activities is not clear. We demonstrate that a

specific connection between Tfb3 and Rpb7 is critical for proper TSS selection. Furthermore, we report the identification of *tfb3* and *tfa1* alleles from genetic screens for putative TSS selection mutants and demonstrate that a subset confers TSS defects in vivo by analysis of the model *ADH1* promoter. These alleles, many of which have residues conserved from budding yeast to human, show TSS shifts at the model promoter *ADH1* and genome-wide (TSS-sequencing). Promoter-level data from our genome-wide TSS mapping provides a quantitative window into phenotypic classes of different initiation mutants. We aim to determine if Pol II-TFIIH interface mutants work with the Pol II active site to promote initiation efficiency or contribute to TFIIH functions and promoter scanning efficiency.

1423S **Identifying the network of genes influenced by the NAD+ dependent deacetylase Sir2 to allow adaptation to low NAD+ stress in the yeast *Kluyveromyces lactis*** Mahasweta Acharjee[1], Kristen Humphrey[1], Vincent Lactaoen[1], Laura Rusche[2] [1]University at Buffalo, [2]State University of New York at Buffalo

Metabolites such as NAD+, which are cofactors in enzymatic reactions, can be synthesized by some species but for other species must be obtained from the environment. For example, most budding yeasts encode *BNA* genes to synthesize NAD+, but *Kluyveromyces* species lack these genes and instead scavenge the precursor, nicotinic acid, from the environment. This project explores how *Kluyveromyces lactis* adapted to the loss of the NAD+ biosynthesis pathway thorough transcriptional rewiring of a gene network repressed by the NAD+-dependent deacetylase Sir2, which is thought to be a sensor of NAD+ availability. We previously compared the genes repressed by Sir2 in *K. lactis* and *Saccharomyces cerevisiae*, which synthesizes NAD+. Interestingly, in *K. lactis,* KlSir2 represses thirteen genes encoding transcription factors, whereas these genes are not repressed by a sirtuin in *S. cerevisiae*. We hypothesized that when these transcription factors are derepressed under low NAD+, they induce additional genes, sustaining and amplifying the response to fluctuating NAD+ levels. To examine how NAD+ levels affect Sir2-regulated gene expression, we grew *K. lactis* cells in varying levels of the precursor nicotinic acid and measured intracellular NAD+ levels and expression of Sir2 target genes. We found that as NAD+ levels decreased, acetylation of histones at target promoters increased, and expression of these genes increased. Interestingly, we found that the drop in NAD+ that is sufficient to induce a transcriptional response is smaller than the drop that affects growth rate. Thus, the cells start responding to falling NAD+ levels early, presumably to buffer against the reduced NAD+. Hence an organism facing stress can modify its gene expression network as a survival strategy before or as soon as its viability and growth is threatened.

1424S **Characterization of Med15-Transcription Factor Interactions and Phase Separation** Shulin Liu[1], David Cooper[2], Emma Grunkemeyer[1], Jan Fassler[1] [1]Biology, University of Iowa, [2]Butler University

Med15 is a glutamine-rich general transcriptional regulator that resides within the tail module of the RNA Pol II Mediator Complex. Strains lacking Med15 are compromised in their ability to grow on many kinds of media, under stress conditions, and in fermentation, reflecting its importance in gene expression. Naturally occurring yeast strains specialized for growth in specific environments (e.g., wine, beer, clinical) vary in their glutamine tract lengths, suggesting that the length of glutamine tracts may influence Med15 function in a manner that is adaptive for a specific environment. The *S. cerevisiae* Med15 protein is one of the most intrinsically disordered subunits of the yeast Mediator Complex and its IDRs likely underlie the role of Med15 as an interaction hub, making contact with a large number of transcription factors. Med15 undergoes liquid-liquid phase separation together with the Gcn4 transcription factor *in vitro*. In this work we map the domain(s) of Med15 that are sufficient for phase separation, examine the contribution of certain amino acid features within that region, and manipulate the length and composition of this region of Med15 to test for a correlation between effects on gene expression and growth and effects on phase separation. In related studies, we examine the nature of the interaction between the different parts of the intrinsically disordered Med15 protein and other transcription factors to determine if phase separation is a general mechanism for Med15 mediated transcriptional activation. Finally, we examine the possibility that the IDRs might also mediate intramolecular interactions. We propose that the IDRs may influence the shape of the Med15 protein, thus indirectly affecting the propensity for phase separation and interactions with transcription factors.

1425S **Induction of budding yeast MFS transporter Tpo1 by anticancer ruthenium complex KP1019 depends on transcription factor Pdr1** Millena Chirillo[1], Lizbett Ocana[1], Hagen Mancuso[2], Pamela Hanson[1] [1]Furman University, [2]Birmingham Southern College

Indazolium *trans*-[tetrachlorobis(1*H*-indazole)ruthenate(III)], also known as KP1019, is an anticancer ruthenium complex with promising anticancer activity in a Phase I clinical trial. KP1019 may serve as an alternative to platinum-based chemotherapies, as it causes fewer side effects. The drug has similar impacts on budding yeast *Saccharomyces cerevisiae* and cancer cells, causing DNA damage and oxidative stress in both. Notably, KP1019 also maintains potency against yeast and cancer cell lines that are multidrug-resistant due to overexpression of ATP-binding cassette (ABC) and/or major facilitator superfamily (MFS) transporters. Thus, yeast can serve as an effective model for studying KP1019.

Published transcriptomic data suggest that KP1019 induces expression of the evolutionary conserved transporter Tpo1 in yeast. Here we verified this drug-dependent induction by using flow cytometry to quantify expression of Tpo1-GFP. Since *TPO1* induction by herbicides and other drugs was previously shown to be controlled by the transcription factor Pdr1, one of the TFs that controls the pleiotropic drug response, we measured the expression of Tpo1-GFP in yeast lacking *PDR1* and/or *PDR3.* Whereas KP1019 treatment increased Tpo1-GFP expression roughly 2-fold in wild-type yeast, compared to untreated controls, deletion of *PDR1* reduced this induction by more than 50%. To verify that *PDR1* is a regulator of *TPO1* expression, we measured Tpo1-GFP levels in yeast with the gain of function allele, *PDR1-11*. The *PDR1* mutant strain displayed a roughly 4-fold increase in Tpo1-GFP levels relative to wild-type controls. Since *PDR1-11* mutants were previously shown to be hypersensitive to KP1019, we examined whether its phenotype was related to *TPO1*. Upon deleting *TPO1* in the *PDR1-11* strain, the tolerance of KP1019 returned to near wild-type levels. Moreover, the deletion of *TPO1* in a wild-type background caused KP1019 resistance.

These results support the hypothesis that KP1019-dependent induction of *TPO1* depends on *PDR1*. Moreover, Tpo1-GFP levels display an inverse relationship with KP1019 resistance, as yeast lacking this transporter are resistant to the drug, but *PDR1-11* yeast that overexpresses *TPO1* are hypersensitive. Given that publicly available datasets show that cancers expressing low levels of MFSD10, the human orthologue of *TPO1*, are resistant to platinum-based chemotherapeutics, we propose that *TPO1* is an evolutionarily conserved modulator of metal drug sensitivity.

1426S **Why are the GABA shunt lower and upper pathway genes regulated differently in *Saccharomyces cerevisiae*?** Terrance G. Cooper[1], Jennifer J. Tate[2] [1]Microbiology, Immunology & Biochemistry, University of Tennessee Health Science Center, [2]Microbiology Immunology and Biochemistry, University of Tennessee Health Science Center

In nitrogen excess, TorC1 phosphorylates Nitrogen Catabolite Repression (NCR)-sensitive transcription activator Gln3, and Tap42, and inhibits charged tRNA-sensitive Gcn2 kinase, and TorC1-bound, phosphorylated Tap42-Sit4/PP2A phosphatase complexes. This results in cytoplasmic Gln3 sequestration and minimal NCR-sensitive transcription. Conversely, nitrogen limitation or starvation down-regulates TorC1 activity, activates Gcn2, permitting Tap42-Sit4/PP2A-dependent Gln3 dephosphorylation, nuclear localization and increased NCR-sensitive transcription. Glutamine levels determine Gln3 binding to its NCR-sensitive target promoters. In low, but not high glutamine, Gln3 must bind to its target promoters before exiting the nucleus. Free Sit4/PP2A dephosphorylate Gln3 exiting from the nucleus in nitrogen excess, whereas Tap42-Sit4/PP2A complexes do so in low nitrogen. Sit4, PP2A and Ure2 are all required to achieve the cytoplasmic Gln3 phosphorylation level. Two Gln3-Torc1 interacting sites exist in Gln3. The C-terminal site functions negatively for sequestering Gln3 in the cytoplasm in excess nitrogen, whereas the N-terminal site responds positively to rapamycin-dependent Gln3 dephosphorylation and nuclear localization. Recent data show Whi2-Psr1/2 suppresses TorC1 activity following a shift from high to low amino acids, but that suppression has little influence on overall NCR-sensitive protein production. This abridged view of Gln3 control applies to how the lower GABA shunt pathway genes are regulated but not the *GAD1* gene encoding the first enzyme in the GABA shunt. However, unlike the lower pathway genes, GAD1 responds to rapamycin inhibited TorC1 kinase but does so independently of Gln3 and Gat1. We demonstrate, using a nickel probe, the integrated, TorC1-dependent, NCR-sensitive-dependent/independent, and GABA-dependent regulation of the upper and lower GABA shunt, retrograde, peroxisomal glyoxylate cycle, and β-oxidation pathways and distinct functions of Gad1. Supported by Harriet S. VanVleet Chair of Excellence and NIH grant GM35642-27.

1427S **Chromatin-mediated hypoxic stress response by Set4 in *S. cerevisiae*** Winny Sun, Yogita Jethmalani, Maraki Negesse, Isabella Justice, Shandon Amos, Erin M Green University of Maryland, Baltimore County

Stress responses in yeast are often mediated by changes in gene expression that allow the cells to adapt. This dynamic signaling occurs through changes in transcriptional regulation, often dependent on the chromatin environment. Set4 is a chromatin-associated protein that is protective in hypoxic stress, during which its expression is induced. Set4 is part of the SET domain family of lysine methyltransferases but appears to lack catalytic activity. Besides its paralog Set3 in yeast, there are other orthologs that share this feature, including in worms, fruit flies, and mammals.

The mechanism by which Set4 promotes survival under hypoxic stress has yet to be determined. Set4 regulates expression of subtelomeric genes, including stress response genes and cell wall components such as the *PAU* family, which are known to be highly induced under hypoxia. Here, we will present our current investigation on Set4 interactions with chromatin modifiers and transcription regulators at stress response genes. We have shown that loss of *SET4* leads to changes in local chromatin environment in hypoxia, including an increase in acetylation at H3K9 at the *PAU* promoters where Set4 localizes during hypoxic stress. This correlates with our observation at these regions that loss of *SET4* affects the localization of the histone deacetylase Rpd3, which can deacetylate H3K9. We also investigate the effect of *SET4* on the association of histone acetylase Gcn5, a catalytic subunit of the SAGA chromatin remodeling complex, which can acetylate H3K9 and has been shown to

regulate *PAU* expression during hypoxia.

Upc2 is a transcription factor that induces *PAU* gene expression upon the switch from aerobic to hypoxic conditions, as well as the induction of *SET4* expression, which then represses *PAU* expression. We have shown that in the absence of *SET4*, *PAU* gene expression is derepressed under hypoxia, and this is dependent on the presence of *UPC2*. Additionally, we show using chromatin immunoprecipitation that *SET4* affects the association of Upc2 to *PAU* promoters.

Altogether, this work provides insight into chromatin regulators that work with Set4 to control gene expression in response to hypoxia and identifies new chromatin regulatory pathways that may also function more broadly to control stress responses in other organisms.

1428S **A Novel Transgenic Reporter to Study Vertebrate Epigenetic Reprogramming During Wound Healing and Regeneration** Jian Ming Khor, Aniket V Gore, Kiyohito Taimatsu, Miranda Marvel, Daniel Castranova, Allison Goldstein, Gennady Margolin, Brant M Weinstein National Institutes of Health

Epigenetic reprogramming plays a pivotal role in reactivation of otherwise silent developmental programs during wound healing and regeneration in adults. Unlike most vertebrate model organisms, zebrafish possess the ability to faithfully regenerate many complex organs and appendages throughout their lifespan. However, the mechanisms regulating the epigenetic changes that permit robust regeneration following injury have yet to be elucidated. This is in part due to the lack of tools for studying epigenetic changes in a tissue- and cell-specific manner. We have generated an “EpiTag” zebrafish transgenic reporter line that permits easy and rapid visualization of tissue-specific loss or gain of epigenetic silencing in living animals at cellular resolution. We observe early and dramatic activation of the EpiTag reporter in regenerating cells of many different tissues including amputated caudal fin rays and cryo-injured hearts and skeletal muscle. We have already used single-cell RNA-seq of regenerating caudal fins at various time to show that multiple cell types undergo major shifts in their global gene expression patterns during fin regeneration. We are currently studying the epigenetic changes taking place in regenerating cells using the expression of the EpiTag reporter as a marker to highly enrich for actively reprogramming cells at the very earliest stages of regeneration. The ability to isolate and study these cells opens the door to important insights into the role of epigenetic reprogramming within the broader context of regenerative processes.

1429V **Targeting Regulatory Factors Associated with the *Drosophila Myc cis*-Regulatory Modules (Myc-CRMs) by Reporter Activity Study, Gel Shift Assay, and Mass Spectrometric (MS) Analysis** Jasmine Kharazmi[1], Cameron Moshfegh[2], Thomas Brody[3] [1]Reproductive Endocrinology, [2]Department of Health Sciences and Technology, ETH Zurich, Laboratory of Applied Mechanobiology, [3]National Institutes of Health (NIH)

Transcription factor MYC is highly responsive to mitogenic stimuli to modulate the expression of several targets involved in cell growth, protein biogenesis, proliferation, and differentiation. However, the regulation of MYC at the level of transcription is still incompletely understood. In previous work, we showed that most of the regulatory elements associated with *Drosophila Myc* are located within the 5’ UTR and the intergenic regions of *Myc*. In this study, we developed a highly sensitive and selective Solid Surface Magnetic Enrichment Protocol (SSMEP) and used it to enrich DNA-Protein complexes compounded with the *Myc cis*-elements. Using smaller truncations, Electrophoretic-Mobility Shift Assay (EMSA) and protein identification by Mass Spec, we identified a cis-regulatory module (CRM) within the proximal 5’-UTR that might be sufficient to activate lacZ reporter in the ovary and embryos. Furthermore, each of the CRMs from the upstream or In2 region in conjunction with the proximal 5’-UTR regulatory unit recapitulates the missing expression in the larval tissues, the ovary, and in embryos. Finally, the Downstream Promoter Element (DPE) shows promoter activity and mimics the *Myc* wild type expression when the adjacent cluster of binding sites is fused to in, in frame to it. *In vivo* functionality tests can be conducted to confirm the relevance of associated factors and identified signaling cascades as *Myc* regulators. In addition, the highly selective and sensitive protocol can be used for other model organisms and biomedical research projects and/or synthetic biological studies.

1430V **Interplay between the Bithorax complex and Wnt signaling in regulating lipid homeostasis in *Drosophila*** Rajitha Udakara Sampath Hemba-Waduge[1], Mengmeng Liu[1], Xiao Li[2], Jasmine Sun[1], Elisabeth Budslick[1], Jun-Yuan Ji[1] [1]Biochemistry and Molecular Biology, Tulane University School of Medicine, [2]Lewis-Sigler Institute of Integrative Genomics, Princeton University

Adipocytes are distributed throughout the human body, playing critical roles in lipid metabolism and energy homeostasis. Notably, excessive visceral fat in the abdominal cavity poses a higher risk of diseases such as diabetes, cardiovascular diseases, and various cancers compared to subcutaneous fat elsewhere. However, the mechanisms governing the regional differences of adipocytes remain poorly understood. Using *Drosophila* as a model organism, we discovered a novel connection between the bithorax complex (BX-C) and the Wingless/Wnt signaling pathway in regulating the regional heterogeneity in larval adipose tissue. The *Drosophila* BX-C comprises three homeotic genes - *Ultrabithorax* (*Ubx*), *abdominal A* (*abd-A*), and *Abdominal B* (*Abd-B*). While the BX-C proteins are known for their roles in regulating the embryonic segmental body

plan, their functions in larval adipocytes have been unexplored. Our research has revealed a distinct heterogeneity between larval adipocytes in the abdominal and thoracic regions, with additional heterogeneity within abdominal adipocytes. We have observed that *abd-A* and *Abd-B* are predominantly expressed in the abdominal adipocytes, exhibiting heterogeneous expression patterns among these adipocytes. Depleting BX-C genes in larval adipocytes resulted in reduced fat accumulation, delayed larval-pupal transition, and ultimately pupal lethality. Furthermore, active Wnt signaling amplified this adipocyte heterogeneity, while depletion of either Abd-A or Abd-B reduced the Wnt-activated gene expression and mitigated the adipocyte heterogeneity induced by active Wnt signaling. These findings highlight the intricate interplay between the BX-C proteins and Wnt signaling in defining adipocyte heterogeneity and regulating lipid homeostasis in *Drosophila*.

1431V **Utilizing Live Cell Imaging in Drosophila melanogaster Salivary Glands to Determine if Resveratrol Treatment Activates Heat Shock Factor DNA Binding** Martin Buckley[1], Tyra Skalos[1], Nichole Webb[1], Stacy Hrizo[2] [1]Slippery Rock University of Pennsylvania, [2]Biology, Slippery Rock University of Pennsylvania

One major stress response pathway is the heat shock response (HSR), which is mediated by the transcription factor, heat shock factor (HSF). The HSR is activated in cells exposed to conditions that induce protein misfolding, such as: high heat, oxidants, and other chemical stresses. Under such stressors, HSF activates expression of the Hsp70 chaperone, which helps cells deal with protein folding stress. However, HSR activation also leads to an increase in reactive oxygen species (ROS), which can damage cellular molecules. To combat this, cells are known to utilize endogenous antioxidants to scavenge free radicals through redox reactions. Therefore, we previously examined the effect of feeding an exogenous antioxidant, resveratrol, on the ability of wildtype Drosophila to withstand heat stress. Treatment with 100uM and 400uM resveratrol increased the ability of the flies to withstand heat stress-induced paralysis. We hypothesize that this result occurred because the flies had increased HSF activity due to the resveratrol treatment. To examine this hypothesis, Drosophila larvae expressing HSF-GFP were dissected to obtain salivary glands. These glands contain large polytene chromosomes that allow for visualization of HSF chromosomal binding using confocal microscopy. The most easily visible binding site is an HSF doublet binding at the Hsp70 loci. Salivary glands at room temperature function as a non-heat shock (NHS) control and exhibit no binding of HSF-GFP at the Hsp70 loci. Salivary glands heated to 37C for 10, 20, 40 minutes function as the positive control and exhibit the expected Hsp70 doublet from HSF-GFP binding of the DNA. We are testing variable concentrations (100uM, 200uM, and 400uM) of resveratrol dissolved in 0.5% DMSO to determine if it activates HSF-GFP binding of the DNA in salivary glands under non-heat shock conditions. Our preliminary data indicates resveratrol treatment does not lead to the recruitment of HSF at HSP70 loci in living polytene nuclei. Follow-up experiments are currently underway to examine the levels of Hsp70 protein in cells treated with resveratrol.

1432V **Neuroprotective Potential of Hydroalcoholic Extract of Centella asiatica Against 3-Nitropropionic Acid-Induced Huntington's Like Symptoms in Adult Zebrafish** Nitasha Rana Pharmacology, ISF College of Pharmacy, Moga

Huntington's disease (HD) is an inherited neurodegenerative disease. 3-Nitropropionic acid (3-NP) causes increased reactive oxygen species production and neuroinflammation. Centella asiatica (CA) is a strong antioxidant. The aim of this study is to investigate the effect of hydroalcoholic extract of C. asiatica (HA-CA) on 3-NP-induced HD in adult zebrafish. Adult zebrafish (*5–6 months old) weighing 470 to 530 mg was used and treated with 3-NP (5 mg/kg intraperitoneal [i.p.]). The animals received HA-CA (80 and 100 mg/L) daily for up to 28 days in water. Tetrabenazine (3 mg/kg i.p.) was used as a standard drug. We have done an open field test (for locomotor activity), a novel tank diving test (for anxiety), and a light and dark tank test (for memory), followed by biochemical analysis (acetyl-cholinesterase [AchEs], nitrite, lipid peroxidation [LPO], and glutathione [GSH]) and histopathology to further confirm memory dysfunctions. 3-NP-treated zebrafish exhibit reductions in body weight, progressive neuronal damage, cognition, and locomotor activity. The HA-CA group significantly reduced the 3-NP-induced increase in LPO, AchEs, and nitrite levels while decreasing GSH levels. Oral administration of HA-CA (80 or 100 mg/L) significantly reduces 3-NP-induced changes in body weight and behavior, in addition to neuroinflammation in the brain by lowering tumor necrosis factor-a and interleukin-1b levels. Moreover, HA-CA significantly decreases the 3-NP-induced neuronal damage in the brain. HA-CA ameliorates neurotoxicity and neurobehavioral deficits in 3-NP-induced HD-like symptoms in adult zebrafish.

1433V ***puf-9* promotes clearance of miR-35-41 microRNAs and somatic sex determination in *C. elegans*** Amelia F Alessi[1], Danny Yang[2], Mallory A Freeberg[2], Margaret R Starostik[1], John K Kim[1] [1]Biology, Johns Hopkins, [2]University of Michigan - Ann Arbor

MicroRNAs (miRNAs) are a conserved group of ~21nt RNAs that regulate post-transcriptional gene expression. MiRNA regulation is essential for diverse biological processes including differentiation and development, stress response, behavior and longevity. Dysregulation of the miRNA pathway has been linked to cancer and cardiovascular and metabolic disorders. Thus, understanding how miRNAs are regulated is critical to fully understanding their function. In *C. elegans,* the miR-35-42

family (miR-35Fam) is required for somatic sex determination (SSD), viability and fertility. miR-35Fam is highly expressed in the early embryo, where it regulates SSD by preventing precocious expression of *sup-26* and *nhl-2* (McJunkin 2014 *G3;* McJunkin 2017 *G&D).* Later in embryogenesis, the miR-35Fam is rapidly cleared by an incompletely understood mechanism. To explore regulation of miR-35Fam, we performed a targeted RNAi screen and assessed miR-35 levels in the 1st larval stage (L1d). We identified *puf-9* as a positive regulator of miR-35Fam clearance. Mutant analysis confirmed *puf-9(-)* have ~10-fold more miR-35 in L1d than wild-type. Additionally, *puf-9(-)* have enhanced SSD defects in *her-1(n695)* gain-of-function animals, indicating that *puf-9* promotes SSD. *her-1* mRNA is also ~2.5 fold upregulated in *puf-9(-)* in conjunction with ~8 fold upregulation of *mir-35-41*-dependent pseudomale genes. PUF-9 is part of the conserved Pumilio/FBF RBP family that regulates mRNAs by translational repression/degradation. To determine how *puf-9* promotes miR-35Fam clearance and SSD, we identified mRNAs bound by PUF-9 in embryo and L1d. Like other PUF proteins, PUF-9 preferentially binds the 3'UTR of mRNAs at UGUA-containing motifs, including *sup-26* and *nhl-2*. Thus far, we have established that *sup-26* and *nhl-2* are both required for *puf-9*-dependent miR-35 clearance and SSD genetic and molecular defects. Interestingly, *puf-9(-)* exhibit only modest upregulation of *sup-26* or *nhl-2* mRNA levels, suggesting that PUF-9 may regulate *sup-26* and/or *nhl-2* via translational repression. We are currently investigating if SUP-26 and NHL-2 protein levels are altered in *puf-9(-)* and whether PUF-9 directly regulates *sup-26* and *nhl-2* via our identified binding sites. Taken together, we propose that PUF-9 and the miR-35Fam co-regulate *sup-26* and *nhl-2* to ensure robust timing of an essential embryonic developmental program and that *puf-9* promotes miR-35Fam clearance through a target-dependent feedback mechanism.

1434V **The loss of SWI/SNF factor ARID1A leads to a cold tumor phenotype via suppression of IFN-g in neuroblastoma** Pamela Watson, Chelsea DeVaux, Kevin Freeman Genetics, Genomics, Informatics, University of Tennessee Health Science Center

Neuroblastoma (NB) is a highly aggressive, heterogeneous disease that arises from the sympathetic nervous system (SNS) and originates from neural crest cells (NCCs). NB is the most common extracranial cancer in pediatric patients, also accounting for 15% of the total number of pediatric cancer deaths. This high mortality rate is the result of the metastatic, immune evasive, and treatment resistant characteristics of the disease, as well as its heterogenous nature. It has been proposed that NB arises from blocks in differentiation during development, resulting in a mixture of two distinct cell types within the tumor: mesenchymal and adrenergic. The adrenergic cell type is more differentiated, and patients that exhibit more of this cell type have greater treatment success. The mesenchymal cell type is more stem like and is associated with the metastatic, immune evasive, and treatment resistant traits of NB. Because NB is considered a disease of development, many therapeutic strategies targeting differentiation exist for patients. Our work is currently focused on interrogating how the loss of ARID1A, a component of SWI/SNF, affects neuroblastoma's (NB) oncogenesis, its contribution to the heterogeneous cell populations in NB, and the immune evasive effects of the mesenchymal cell type.

Recent data reported that ARID1A has a role in initiating inflammatory signaling in adult cancers by opening areas of chromatin thereby enabling the transcription of Th1 signaling genes, but this immune impairment has not been sufficiently investigated in NB. NB is considered a cold tumor, a designation characterized by a lack of infiltrating CD8+ T cells in the tumor microenvironment, low MHC-I expression, and lack of tumor antigens. All the cold tumor characteristics further decrease therapeutic options for patients, and the few options that are available can lead to severe toxicities and adverse events. All of this suggests that differentiation of the immature, mesenchymal cell types could expand therapeutic options for treatment resistant cells including immunotherapy options in the future. Previously, the Freeman lab established that ARID1A loss, not MYCN amplification, is the root cause for immaturity in NB; we now want to establish ARID1A loss as a key factor for the cold tumor type in NB. Thus, we will interrogate the role of ARID1A in inflammatory signaling and immune infiltration as well as the effects of its loss within NB.

1435T **Identifying suppressors of stress-induced neurodegeneration in a *C. elegans* knock-in *sod-1 G85R* ALS model** Mika Gallati[1], Katherine S. Yanagi[2], Alexander T. Lin-Moore[2], Anne C. Hart[2] [1]Neuroscience, Brown University, [2]Brown University

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease mainly affecting cholinergic and glutamatergic motor neurons. Despite the discovery of several causal mutations in key genes, convergent cellular processes of ALS-associated neurodegeneration have still not been identified. Therefore, determining genetic modifiers of neurodegeneration in SOD1 ALS models can give insight into the mechanisms of ALS-associated neurodegeneration. Previously, the Hart lab generated a single-copy knock-in model of SOD1 G85R, a causative allele of ALS, in the model nematode *C. elegans*. Introduction of SOD1 G85R leads to specific degeneration of glutamatergic and cholinergic neurons following exposure to oxidative stress (PMID: 30296255). With this model, we performed a forward genetic screen to identify suppressors of neurodegeneration by exposing the *sod-1 G85R* model to ethyl methyl sulfonate (EMS). Lines were screened by assessing the suppression of neurodegeneration in glutamatergic tail neurons (12,586 haploid chromosomes screened). Lines showing suppression were isolated, ranging from slight decreases in degeneration to near-complete rescue. I have backcrossed the three lines with

the strongest suppression to diminish the number of background mutations caused by EMS and to create sibling lines that do not contain the causal mutation. I sent these lines for whole genome sequencing to identify causal mutations via the sibling subtraction method (PMID: 29237702). Through the use of MiModD, I garnered a list of possible causal mutations by subtracting mutations detected in sibling lines from those in the suppressor lines. This analysis pipeline is currently being implemented for additional suppressor lines identified from the screen. Identification of these suppressor genes may improve our understanding of the mechanisms underlying ALS and potentially inform the development of future treatments.

1436T **Inducible deletion of the *Caenorhabditis elegans* Alzheimer's-related gene *apl-1* demonstrates temporal restriction of essential functions** Megan J Moerdyk-Schauwecker, Christine Sedore, Zachary Munoz, Erik Segerdell, John Willis, Patrick Phillips University of Oregon

Aggregation of amyloid beta, a peptide derived from amyloid precursor protein (APP) is a diagnostic feature of Alzheimer's disease. However, while the role of the APP family of proteins - which is evolutionarily conserved across a number of vertebrates and invertebrates - is established in development, their non-pathogenic functions post-development are less clear. *Caenorhabditis elegans* has a single member of the APP family, *apl-1*. Since *apl-1* is developmentally required in *C. elegans*, we developed a conditional gene deletion system to study post-developmental function. The native *apl-1* gene was replaced with a cassette containing *apl-1* flanked by lox sites as well as a fluorescent reporter system that allowed *apl-1* removal - triggered by inducible expression of Cre - to be monitored at the level of individual cells based on a green-red fluorescent shift. We also took advantage of the ability of Cre fragments to self-assemble to delete *apl-1* in both a temporal and tissue specific manner, albeit with lower efficiency. A similar system could also be employed for the conditional deletion of other genes. While we were able to recapitulate the embryonic lethality phenotype in our system, early life deletion of *apl-1* did not reduce lifespan in *C. elegans*. Furthermore, while whole worm RNA-seq confirmed knock-down of *apl-1* transcripts, expression of all other genes was unchanged. Thus *apl-1* appears unnecessary, or at least required at much lower than typical physiological levels, for normal function in post-developmental *C. elegans*.

1437T **Mutation in F-actin polymerization factor suppresses Distal Arthrogryposis Type 5 (DA5) PIEZO2 pathogenic variant in *Caenorhabditis elegans*** Xiaofei Bai[1], Harold E. Smith[2], Valeria Vasquez[3], Luis O Romero[4], Briar Bell[4], Andy Golden[2] [1]Biology, University of Florida, [2]National Institutes of Health, [3]Biochemistry and Molecular Biology, Center for Membrane Biology, the University of Texas Health Science Center at Houston McGovern Medical School, [4]Center for Membrane Biology, the University of Texas Health Science Center at Houston McGovern Medical School

The mechanosensitive PIEZO channel family has been linked to over 26 disorders and diseases. Although progress has been made in understanding these channels at the structural and functional levels, the underlying mechanisms of PIEZO-associated diseases remain elusive. In this study, we engineered four PIEZO-based disease models using CRISPR/Cas9 gene editing. We performed an unbiased chemical mutagen-based genetic suppressor screen to identify putative suppressors of a conserved gain-of-function variant *pezo-1[R2405P]* that in human *PIEZO2* causes distal arthrogryposis type 5 (DA5; p. R2718P). Electrophysiological analyses indicate that *pezo-1(R2405P)* is a gain-of-function allele. Using genomic mapping and whole-genome sequencing approaches, we identified a candidate suppressor allele in the *C. elegans* gene *gex-3.* This gene is an ortholog of human *NCKAP1*(NCK-associated protein 1), a subunit of the Wiskott-Aldrich syndrome protein (WASP)-verprolin homologous protein (WAVE/SCAR) complex, which regulates F-actin polymerization. Depletion of *gex-3* by RNAi, or with the suppressor allele *gex-3(av259[L353F])*, significantly restored the small brood size and low ovulation rate, as well as alleviated the crushed oocyte phenotype of the *pezo-1(R2405P)* mutant. Auxin-inducible degradation of GEX-3 revealed that only somatic-specific degradation of GEX-3 restored the reduced brood size in the *pezo-1(R2405P)* mutants*.* Additionally, actin organization and orientation were disrupted and distorted in the *pezo-1* mutants. Mutation of *gex-3(L353F)* partially alleviated these defects. The identification of *gex-3* as a suppressor of the pathogenic variant *pezo-1(R2405P)* suggests that the cytoskeleton plays an important role in regulating PIEZO channel activity and provides insight into the molecular mechanisms of DA5 and other PIEZO-associated diseases.

1438T **Age-related changes in the morphology of a single-cell stem cell niche** Nilay Gupta[1], E. Jane Albert Hubbard[2] [1]Developmental Genetics, NYU Department of Biology, [2]Cell Biology, NYU Grossman School of Medicine

Morphological characteristics of cells often correspond to specific functions. Cells that participate in direct cell-cell contact and contact-dependent intercellular signaling may be especially reliant on their morphology. Age-dependent alterations in cell shape could conceivably lead to pathologies resulting from impaired function and/or signaling. How organismal age impacts cell morphology is not well understood. To understand how aging affects cell morphology, we are studying the distal tip cell (DTC) in the adult *C. elegans* hermaphrodite. The DTC produces membrane-bound ligands for the germline-expressed Notch receptor, GLP-1, the activity of which specifies germline stem cell fate. The adult hermaphrodite DTC has a complex cellular morphology that includes long proximally-extending processes (Byrd *et al.*, 2014). Previous work indicated that the germline

stem cell pool becomes depleted with age (Qin and Hubbard, 2015; Kocsisova *et al.*, 2019), and that *daf-2* (insulin/IGF-like receptor) mutants resist this depletion in a manner dependent on the DAF-16 (FOXO) transcription factor and on germline flux. Qin and Hubbard (2015) also showed that *daf-16* activity is required germ cell non-autonomously in the proximal somatic gonad to control the adult progenitor pool with age, distinct from its role in regulating lifespan. Here, using live imaging of the DTC, we show that three distinct DTC morphology parameters radically change with age. Surprisingly, we find that these parameters are differentially responsive to changes in *daf-2* and *daf-16* activity: nuclear displacement is independent of *daf-2*, the number of long processes is sensitive to *daf-2* but not to *daf-16*, and the length of processes is sensitive to both *daf-2* and to *daf-16*. Further, our results suggest that *daf-16* regulates DTC morphology non-autonomously from the muscle, a tissue requirement that is distinct from the influence of *daf-16* on the aging germline stem/progenitor pool and distinct from its influence on longevity. Our results offer a new model for the effects of aging on cell morphology, demonstrate *daf-2*-dependent and -independent aspects of aging cellular morphology, identify the muscle as a source of *daf-16*-dependent regulation of aging DTC morphology, and underscore the necessity to understand the regulation of age-dependent changes in cell morphology at the level of the whole organism.

1439T **Systematic creation and rapid phenotyping of Mendelian disease models in *C. elegans*: towards large-scale high-throughput drug repurposing** Thomas J O›Brien[1,2], Andre EX Brown[1,2] [1]Faculty of Medicine, Imperial College London, [2]MRC Laboratory of Medical Sciences

The goal of precision medicine is to tailor treatments for individuals based upon genomic sequencing data. Advancements in genome sequencing mean new Mendelian diseases are constantly discovered, however the majority of genetic disorders have no approved treatments. This is particularly true for rare disorders, where the identification of a genetic lesion may not lead to a candidate target because the cause is a complete loss of function of the causal gene or there is little understanding of the genetic pathways implicated. To address this need, we require scalable approaches that are relatively inexpensive compared to traditional drug development. In the absence of a validated drug target, phenotypic screening in model organisms provides a route for identifying candidate treatments. Success requires a screenable phenotype, however the right phenotype and assay may not be obvious for pleiotropic neuromuscular disorders.

We have developed tracking technologies capable of measuring changes in behaviour as the result of genetic mutation, or treatment with small molecules/metabolites. To test the power of our approach in treating human genetic disorders, we generated a panel of *C. elegans* strains containing mutations orthologous to those associated with rare human neuromuscular disorders. These strains represent patient-specific 'avatars' of rare genetic disease. Despite some mutations causing phenotypes imperceptible to the naked eye, they are captured by quantitative phenomics. The resulting 'behavioural fingerprints' provide a high-content view of multidimensional phenomic space for individual genetic disorders. With these, we have performed high-throughput screens of drug libraries and identified novel repurposing candidates with the potential to treat rare genetic disorders with no prior knowledge of the molecular basis of disease.

Our results show how a single assay, measuring multiple phenotypes, can be applied systematically to diverse Mendelian disease models. The relatively short time and low cost associated with creating and phenotyping multiple strains suggests that high-throughput worm tracking could provide a scalable approach to drug repurposing commensurate with large numbers of Mendelian diseases. Ultimately, this work will enable the development of a high-throughput precision medicine platform to identify candidate compounds for the treatment of many genetic disorders, using an individual's personalised *C. elegans* disease-model avatar.

1440T ***Stenotrophomonas indicatrix* promotes innate immune response against intracellular pathogens in *Caenorhabditis elegans*** Jordan D West, Samuel Li, Vladimir Lazetic Biological Sciences, The George Washington University

How do changes in intestinal microbiome affect innate immunity in *Caenorhabditis elegans*? To answer this question, we are studying the Intracellular Pathogen Response (IPR), which is a robust transcriptional response that protects *C. elegans* against obligate intracellular pathogens such as the Orsay virus and fungus *Nematocida parisii*. The IPR is activated upon infection and involves the upregulation of about 80 genes, some of which encode Cullin-RING ubiquitin ligase components. In this study, we performed a screen using different bacterial species isolated from the natural habitats of *C. elegans* and tested if any of them could induce the IPR in the absence of infection. We found that *Stenotrophomonas indicatrix* (JUb19) significantly enhanced the expression of the IPR reporter, *pals-5*p::GFP, when compared to the standard bacterial food source *Escherichia coli* (OP50). Our data indicate that the presence of alive JUb19 is necessary for IPR induction, as heat inactivation of bacteria suppressed the phenotype. Following JUb19 treatment, the expression of *pals-5*p::GFP reporter was observed in many tissues including the intestine, epidermis, and neurons, as well as several tissues where it had not been reported before – coelomocytes and somatic gonad. Our findings suggest that JUb19 treatment leads to a developmental delay which is a phenotype that has been previously associated with constitutive IPR activation. Animals fed with JUb19 also displayed lower infection levels than OP50-

fed animals, indicating that JUb19 protects against intracellular pathogens. In summary, our findings establish JUb19 as the first bacterial species that can induce the IPR in *C. elegans*.

1441T **Synthetic multivulva (SynMuv) genes and mes-4 antagonistically regulate the Intracellular Pathogen Response (IPR) in Caenorhabditis elegans** Samuel Li[1], Eillen Tecle[2], Theresa Bui[2], Michael J Blanchard[2], Emily R Troemel[2], Vladimir Lazetic[1,2] [1]Biological Sciences, The George Washington University, [2]Cell and Developmental Biology, University of California, San Diego

How do non-professional immune cells of the nematode Caenorhabditis elegans respond to natural intracellular pathogens? To answer this question, we are studying the Intracellular Pathogen Response (IPR) – a transcriptional response in C. elegans, that is activated by two molecularly distinct pathogens: Orsay virus and microsporidia Nematocida parisii. The IPR consists of 80 highly upregulated genes, including those encoding cullin-RING ubiquitin ligase components. Previous studies have shown that constitutive activation of the IPR protects against intracellular pathogens and improves proteostasis. Induction of the IPR also shares several similarities with the type-I interferon response in mammals. Through two independent forward genetic screens, we identified a member of the synthetic multivulva (SynMuv) family, lin-15b, as a negative regulator of the IPR. lin-15b, in conjunction with other SynMuv genes, are known regulators of development in C. elegans. Here, we found that loss of lin-15b causes constitutive expression of the fluorescent IPR reporter, upregulation of IPR genes, and resistance to N. parisii infection in the intestine. In a subsequent RNAi screen, we found that many other SynMuv genes, including components of DRM, NuRD, and SUMOylation complexes, control the IPR reporter expression. Finally, we found that IPR upregulation in lin-15b mutants is dependent on histone methyltransferase MES-4, which is a critical regulator of chromatin remodeling in embryonic and germline tissues. Previous studies have demonstrated opposing functions of some SynMuv genes and mes-4 in transcriptional regulation and chromatin remodeling during embryonic development. Our study suggests that antagonism between SynMuv genes and mes-4 is not restricted to embryonic development and that it plays an important role in regulating intestinal immune responses in C. elegans.

1442T **Impact of Stress on the Notch Signaling Pathway in *C. elegans*** Amy Hebert, Ismael Curiel Elmhurst University

At one point or another, we have all felt the strain that stems from stress, whether it be school, work, or other life stressors. There can be many physiological impacts such as long-term stress leading to neuron death in the hippocampus which could result in a decreased learning and memory. Utilizing *C. elegans* as a model organism, we assessed the impacts of temperature stress on learning and memory. Previous work in our lab has shown that temperature stress exposure (28°C) for 3 days during development leads to a significant reduction in learning and memory as measured by a chemotaxis assay. In this current work, we are aiming explore potential mechanisms for this reduction and are looking into the Notch signaling pathway that our previous work has shown is significantly impacted, along with learning and memory, in *C. elegans* when exposed to inflammatory cytokines. *C. elegans* that are GFP-tagged at adm-4, a key regulator of the Notch1 signaling pathway, were age matched and exposed to 28°C stress for 3 days before being analyzed for average fluorescence at the nerve ring. Change in average fluorescence allows us to gain an understanding of the impact that stress has on the regulation of the Notch signaling pathway and how this could be contributing to declines in learning and memory that are seen during neurodegenerative diseases such as Alzheimer's disease.

1443T ***Caenorhabditis* Intervention Testing Program: Updates on robust longevity effects of novel compounds in genetically diverse nematodes** Monica Driscoll[1], Christine Sedore[2], Anna Coleman-Hulbert[2], Erik Johnson[2], Erik Segerdell[2], Brian Onken[1], David Hall[3], Theresa FitzGibbon[3], Yuhua Song[1], Madhuri Achanta[1], Anna Foluger[3], Mustafa Sheikh[3], Stephen Banse[2], Gordon Lithgow[3], Partick Phillips[2] [1]Rutgers, The State University of New Jersey, [2]University of Oregon, [3]Buck Institute for Research on Aging

Many efficacious interventions that promote mouse longevity (sirtuins, TOR, insulin signaling) have identification roots in invertebrate genetics. The *Caenorhabditis* Intervention Testing Program (CITP) was charged by the National Institute on Aging to evaluate pharmacological interventions that promote healthy aging in a robust and reproducible manner across diverse genetic backgrounds of natural variant *Caenorhabditi*s strains. The central premise of the CITP effort is that compounds that have strong effects across diverse genetic backgrounds should have enhanced probability of translatability into pre-clinical research. Indeed, some CITP-verified compounds have been shown to promote healthspan and longevity in mouse models, in support of this fundamental premise.

Our current effort includes pursuing compound submissions from the larger scientific community of researchers on aging biology, as well as identification of candidates via high-throughput screening of chemical libraries and data mining of peer-reviewed publications. We now evaluate whole-organism RNA sequence data to estimate the mode of action for successful interventions, and conduct mortality analysis from a generalized family of distributions on high-resolution automated lifespan

data to determine whether a given intervention changes the rate or onset of aging. We are also creating multi-species “at risk” test sets, in which components of aging hallmarks (such as proteostasis, stress response circuits) are genetically compromised in diverse genetic backgrounds. Such genetic test sets might expand understanding of intervention action and help rank compounds for translational testing.

Recent promising CITP longevity interventions include a small molecule, a proprietary compound, an isothiocyanate and a vitamin derivative. In addition, we find that metabolic modulator Metformin and anti-amyloid Thioflavin T, which promote longevity and healthspan in multiple test CITP strains, can positively impact lifespan in diverse genetic backgrounds in which proteostasis is compromised, suggesting wide-ranging health benefits of these potent pro-longevity compounds. We will present on the breaking compound successes.

1444T **The impact of over-supplementation with folic acid after hypoxia in *Drosophila melanogaster*** Siddarth Gunnala[1], Alisha Harrison[2], Amber Juba[2], Paula Ashcraft[3], Teodoro Bottiglieri[3], Lori M Buhlman[2,2], Nafisa M Jadavji[1] [1]Biomedical Sciences, Midwestern University, [2]Midwestern University, [3]Baylor Scott and White Research Institute

Hypoxia is a major component of ischemic stroke, which is known for its disabling prognosis. Nutrition is a risk factor for the onset of ischemic stroke, as well as outcomes after a stroke. Our previous work, along with other published literature has shown the positive impact that folic acid supplementation can have after ischemic stroke. Folic acid is a water-soluble B-complex vitamin (B9) involved in nucleic acid synthesis, methylation, and DNA repair. It plays a central role in one-carbon (1C) metabolism, which is a metabolic network that integrates nutritional signals with biosynthesis, redox homeostasis, and epigenetics. Recently, over-supplementation of folic acid has become a problem in many countries with mandatory food fortification laws in place to reduce the prevalence of neural tube defects. However, the impact of over-supplementation of folic acid on brain injury is not well understood. The aim of our study was to investigate how folic acid over-supplementation impacts hypoxia outcome using *Drosophila melanogaste*r as a model. We maintained w1118 male and female flies on control and 100µM folic acid supplemented diets. Progeny from these parents were exposed to hypoxia (1% oxygen) for a period of two hours after which flies were returned to normoxic conditions to model reperfusion. Using liquid chromatography-tandem mass spectrometry, folic acid and tetrahydrofolate levels were elevated in supplemented flies. We confirmed that flies were exposed to hypoxia by measuring escape latency of larvae from food in vials. The survival rate of flies was recorded after hypoxia treatment for 90 days. In a separate group of flies 24 hours after hypoxia, fly climbing behavior was also measured. This data included the number of movements made and the motivation to climb. We observed that hypoxia treatment increased the number of dead flies over the span of their lifetime in both the control and folic acid supplemented diet (FASD) groups. Furthermore, we observed a reduction in the motivation to fly in control and FASD exposed to hypoxia. We are currently investigating apoptosis in brain tissue of flies to determine potential mechanisms. Preliminary data suggests that folic acid over-supplementation does not exacerbate negative health outcomes after hypoxia using the *Drosophila melanogaster* model.

1445T **Downregulation of Pten suppresses the Huntington’s disease in Drosophila model** Maynglambam Dhruba Singh[1], Nisha Vishwakarma[2], Bhavya Gohil[2] [1]National Brain Research Centre, [2]NBRC

Huntington’s disease (HD) is a dominantly inherited progressive neurodegenerative disorder that affects 3.92 per 100,000 individuals worldwide. The disease is caused by the expansion of CAG repeat in the coding region of Huntingtin gene (HTT). The expanded poly(Q) repeats have propensity to aggerate which induces neuronal cell death. The non-pathogenic CAG repeat length in normal people ranges from 10-35. However, repeats length above 40 is pathogenic and increase in repeat length is inversely correlated with the onset of the disease. Studies have shown that insulin signaling is altered and insulin resistance is induced by mutant Htt protein. We downregulated Downstream of Insulin receptor, Phosphatase and tensin homolog (Pten), in mutant Htt expressing fly model and observed that Pten significantly reduced the HD phenotypes. In the Drosophila eye, knockdown of Pten improved the rough eye phenotype caused by mutant Htt with 138 poly(Q) repeats. The retinal length and photoreceptor neurons were significantly improved. The degeneration of brain neuronal structure in the brain was improved by knockdown of Pten. Knockdown of Pten also improved the integrity of neuromuscular junction (NMJ) in dorso-lateral muscles. In addition, knockdown of Pten significantly improved the functional vision and climbing ability which are affected by mutant Htt138Q. Furthermore, we observed the Knockdown of Pten significantly reduced the number of poly(Q) aggregates in the larval eye disc and antennal lobe of the brain. Moreover, Pten knockdown reduced the level of apoptosis in the larval and pupal eye disc. Our study highlight that Pten is a genetic modifier of Huntington’s disease with potential therapeutic application.

1446T **The structure of human Orc6 protein – humanized Drosophila model of the Meyer-Gorlin syndrome** Maxim Balasov[1], Katarina Akhmetova[1], Guang Zhu[1], Igor Chesnokov[2] [1]University of Alabama at Birmingham, [2]Biochemistry and Molecular Genetics, University of Alabama at Birmingham

Orc6 is an important component of the Origin Recognition Complex (ORC) and has functions in both DNA replication and cytokinesis. We solved a solution structure of the full length human Orc6 alone and in a complex with DNA by NMR methods. We have shown that in eukaryotes, Orc6 has a homology with transcription factor TFIIB. The detailed analysis of the structure revealed amino acid clusters important for DNA binding. We further showed that human Orc6 is composed of three independent domains: N-terminal, middle and C-terminal. A mutation in the conserved C-terminal motif of Orc6 impedes the interaction of Orc6 with core ORC and results in Meier-Gorlin syndrome (MGS). MGS is a rare autosomal recessive disorder characterized by microtia, primordial dwarfism, small ears, and skeletal abnormalities. Patients with MGS often carry mutations in genes encoding the subunits of ORC, components of the pre-replicative complex (pre-RC) and replication machinery. Recently, additional MGS mutations in Orc6 were identified. One mutation is localized in the N-terminal domain of the protein. Another is a compound heterozygous mutation that includes a deletion of 20 amino acids from the N-terminus as well as a mutation within a splice site of the gene potentially resulting in a short deletion in the middle of the protein. Based on the structural information we created a hybrid human-*Drosophila* gene to rescue the *orc6* deletion in *Drosophila* and to study the functions of the protein in a living organism. Using this "humanized" *Drosophila* model of the Meier-Gorlin syndrome we discovered the molecular mechanisms underlying the observed MGS phenotypes. Our studies revealed the importance of evolutionarily conserved and variable domains of Orc6 protein and allowed the studies of human protein functions and the analysis of the critical amino acids in live animal heterologous system. We are expanding these studies to address the importance of Orc6 C-terminal motifs for its interaction with core ORC. We identified a structured motif in the Orc6 C-terminal domain which defines the dynamics of inter-subunit interactions within metazoan ORC. These studies suggest the molecular mechanism for the association of Orc6 with the core ORC in flies and humans.

1447T **An *in vivo* screen identifies small molecule modulators of the endoplasmic reticulum stress response** Emily Coelho, Katherine Beebe, Kevin A. Hope, Alexys R. Berman, Heather D. Evans, Clement Y. Chow University of Utah

Misfolded protein accumulation in the endoplasmic reticulum (ER) induces ER stress. Cells respond to ER stress by initiating the unfolded protein response (UPR) that upregulates chaperone protein expression, increases the degradation of misfolded proteins, and inhibits protein translation. Failure to effectively manage ER stress and restore homeostasis results in cellular dysfunction and ultimately apoptosis, a process implicated in numerous human diseases such as retinal degeneration, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), among others. Identifying small molecules that modulate ER stress may be effective therapeutics for human diseases caused by misfolded protein accumulation. Here, we used a *Drosophila* model of retinitis pigmentosa (RP) that expresses misfolded rhodopsin protein, $Rh1^{G69D}$, in the developing eye. $Rh1^{G69D}$ expression induces chronic ER stress and apoptosis, resulting in a degenerative eye phenotype. We took a drug repurposing approach and used the Prestwick Chemical Library, consisting of 1520 small molecules, the majority of which are FDA-approved, to identify compounds that modulate neuron cell death in $Rh1^{G69D}$ expressing flies. We identified multiple classes of drugs that enhance or suppress the degenerative eye phenotype, including compounds acting through monoamine neurotransmitters, folate metabolism, sodium channels, and the renin/angiotensin pathway. Degeneration-enhancing compounds may reveal novel ER stress pathways, while compounds that suppress degeneration are potential therapeutic candidates for RP. We are using an RNAi approach to identify the mechanism of action for the top enhancers and suppressors. Additionally, we will present data showing that compounds that rescue cell death in the RP model can also rescue disease-associated phenotypes in other *Drosophila* models of protein misfolding diseases, such as PD, HD, and ALS. This work identified potential therapeutic drugs for RP and possibly other human diseases that result from misfolded protein accumulation and ER stress.

1448T **Intake of Caffeine Containing Diet Remodels Gut Microbiota and Perturbs *Drosophila melanogaster* Immunity and Lifespan** Abeer Qush Department of Biomedical Sciences, Qatar University

The diet-microbiome-immunity axis is one among the many arms that draw up the "we are what we intake" proclamation. As such, studies on the effect of food and beverage intake on the gut environment and microbiome and on modulating immunological responses and the host's susceptibility to pathogens are on the rise. A typical accompaniment in different sustenance we consume on daily basis is the *trimethylxanthine* alkaloid caffeine. Being a chief component in our regular aliment, a better understanding of the effect of caffeine containing food and beverages on our gut-microbiome-immunity axis and henceforth on our health is much needed. In this study, we shed more light on the effect of oral consumption of caffeine supplemented sugar diet on the gut environment, specifically on the gut microbiota, innate immunity and host susceptibility to pathogens using the *Drosophila melanogaster* model organism. Our findings reveal that the oral intake of a dose-specific caffeine containing sucrose/agarose sugar diet causes a significant alteration within the fly gut milieu demarcated by microbial dysbiosis and an elevation in the production of *reactive oxygen species and* expression of immune-deficiency (Imd) pathway dependent *antimicrobial peptide* genes*. The oral intake of* caffeine containing sucrose/agarose sugar diet *also renders flies more susceptible to bacterial infection and shortens their lifespan in both infection and non-infection settings.* Our findings set

forth additional insight into the potentiality of diet to alter the gut milieu and highlight the importance of dietary control on health.

1449T **A comprehensive assessment of human huntingtin expression in the nervous system of larval and adult *Drosophila*** Tadros A Hana, Andrew H Michael, Veronika G Mousa, Rawan Haj-Hussein, Madona Aziz, Sabita Basnet, Kiel G. Ormerod Biology, Middle Tennessee State University

Huntington's Disease (HD) is an inherited, monogenic disease affecting ~1 / 10000 people yearly. Mutant Huntingtin protein contains a stretch of glutamine repeats that, when expanded, becomes unstable and forms inclusion bodies associated with the pathophysiology of the disease. Using *Drosophila* 3rd instar larvae as well as adults to model pathogenic huntingtin progression through development and into adulthood. We tracked huntingtin aggregate proliferation from eggs every 24 hours and determined a linear progression in the size and number of aggregates in the developing brain, motor axons, and at the neuromuscular junction. This trend of aggregate proliferation was also observed in the pathogenic huntingtin modeled in the adult wing. Interestingly, larval morphology and viability were not impacted. Using live imaging of fluorescently labeled huntingtin, we determined that axonal aggregates significantly impaired trafficking of organelles like synaptic vesicles, mitochondria, and dense core vesicles. To assess the downstream implications, we conducted electrophysiological recordings from muscles using intracellular voltage recordings and muscle force recordings and observed significant impacts on neuromuscular transduction. Larval crawling patterns were significantly impacted, showing impairments in movement speed and total distance traveled. These physiological deficits were also observed in adult flies, with impairments in motor function as well as behavioral manifestations. The lifespan of adults expressing pathogenic huntingtin was reduced by a third. Novel application of mTOR inhibitor Rapamycin shows a significant increase in lifespan, suggesting a mechanism by which autophagy can influence the pathology linked to huntingtin aggregates. Here we have shown significant molecular, cellular, physiological, and behavioral deficits associated with pathogenic human huntingtin expression in the nervous system of Drosophila. Our work serves as a robust model to assess the progression of HD, and as a rigorous tool for potential genetic and therapeutic treatments for HD disease progression.

1450T ***Prominin-like* regulates feeding behavior through the muscle-brain axis in *Drosophila*** Tae Hoon Ryu, Kweon Yu Disease Target Structure Research Center, Korea Institute of Bioscience and Biotechnology

Energy demands from peripheral tissue affects feeding behavior in animals. Although muscle is one of the most energy-consuming organ, less is known whether muscle-derived hormones regulate feeding behavior. Muscle-derived hormones, also known as myokines, are secreted from muscle in response to nutritional stress or exercise to regulate various physiological function. In this study, we found that *prominin-like* (*promL*), the homolog of Prominin-1, is a regulator of myokine in response to carbohydrates intake. Muscle-specific *promL* inhibition resulted in increased carbohydrate intake while protein intake was not changed. ATP level in the muscle of muscle-specific *promL* inhibition flies was reduced, indicating that *promL* regulates energy metabolism in the muscle. Next, we screened mRNA expression level of myokines in the muscle tissue and found PDGF- and VEGF-related factor 3 (Pvf3) as a feeding regulator. When we inhibited the receptor of Pvf3, PDGF- and VEGF-receptor related (Pvr), using various neuronal Gal4 drivers, Pvr inhibition in serotonergic neurons showed increased food intake. We further showed that the 5-HTP, a precursor of serotonin, rescued food intake and choice behavior, suggesting that Pvf3-Pvr signaling regulates food intake and choice by modulating serotonergic system. Taken together, these findings indicate that *promL* expressed in the muscle mediates feeding behavior by regulating myokine and neurotransmitter signaling.

1451T ***Wolbachia* enhances survival of *Drosophila* infected with fungal pathogens** Jessamyn I Perlmutter, Aylar Atadurdyyeva, Margaret E Schedl, Robert L Unckless University of Kansas

Often called "the world's greatest pandemic", *Wolbachia* bacteria of arthropods are the most widespread endosymbionts on the planet. They are found in over half of all insect species and exist on every continent except Antarctica. These bacteria are vertically transmitted from mother to offspring via the cytoplasm much like mitochondria. To ensure faithful inheritance of the symbiont, *Wolbachia* rely on a set of cunning host interactions to enhance the fitness of hosts carrying the symbiont. Crucially, one phenotype is the ability to inhibit viral pathogens when an arthropod host contains certain *Wolbachia* strains. Mosquitoes with the *w*Mel *Wolbachia* strain of *Drosophila melanogaster*, for example, exhibit significantly reduced transmission rates of viruses like dengue and chikungunya to new hosts. Global initiatives have thus far achieved great success in using *Wolbachia* in the wild to reduce mosquito-borne transmission of viral diseases to humans. However, very few studies have focused on *Wolbachia*'s interactions with other microbes such as fungi despite the prevalence of fungal pathogens in the wild. Here, we perform a comprehensive panel of fungal infection assays with *Drosophila melanogaster* flies with or without *Wolbachia* to fill this gap. We find that the symbiont protects hosts against a wide array of filamentous and yeast pathogens of agricultural and medical importance by increasing host longevity post-infection. Notably, the presence and strength of the phenotype is dependent on factors including host genotype, sex, and fungal species. Flies co-infected with *Wolbachia* and fungus have lower pathogen titers and exhibit higher egg-laying, indicating that the mechanism is likely host resistance and that the phenotype

confers a significant fitness benefit. Further, flies with the symbiont have increased expression of key immune genes post-fungal infection relative to flies without the symbiont, suggesting induction of host immunity may be key to *Wolbachia*'s ability to fight fungal pathogens. This study represents a major advancement in *Wolbachia* research and applications by demonstrating that its famous pathogen-blocking abilities can now be broadly extended to another major branch of microbial life. These results not only provide new information on how *Wolbachia* have achieved such a high global prevalence, but will also inform pathogen-blocking research and open up new possible applications of *Wolbachia* to areas like agriculture.

1452T **Regulation of *Drosophila* intestinal lipid metabolism by the gut microbiota** Joshua Derrick[1,2], Haolong Zhu[1], William B Ludington[3], Steve Farber[1] [1]Biology, Johns Hopkins University, [2]Biology, Carnegie Embryology, [3]Biology, Carnegie Institute of Embryology

Aims and Methods:

Microbiota species associated with western diets have been causally linked to worse health outcomes in humans, although both the mechanism and causal species are unclear. Work in both zebrafish and mice has shown that animals with a microbiome absorb more lipids into their enterocytes from a fatty meal than germ-free animals. In both animals, increased lipid absorption was linked to the presence of specific microbiome species. However, a mechanism has yet to be established: how exactly do microbes promote intestinal lipid absorption. Recent works including ours have developed a fly model with a natural and complete microbiome consisting of up to 5 to 10 members of the *Acetobacter* and *Lactobacilli* clades, which have analogs in the human gut. The goal of my project is to determine if members of this microbial community are linked to increased intestinal lipid absorption or whole animal lipid accumulation, and to establish a mechanistic link between microbial colonization and the altered lipid metabolism of the fruit fly host. Specifically, I used HPLC lipidomics to identify changes in whole animal lipids in the presence and absence of microbiota. To measure the postprandial response, we used the fluorescent lipid BODIPY-C12 as a metabolic tracer that was mixed into fly food containing a non-fluorescent non-absorbable dye to control for feeding rate. Two methods were developed to measure incorporation of this tracer: HPLC with a fluorescence detector was used to measure incorporation of the tracer into complex lipid species, and confocal microscopy was used to measure the spatial distribution and size of lipid droplets in the drosophila gut. Additionally, I developed a luminescent reporter line for the drosophila lipoprotein ApoLpp (a gene with significant homology to mammalian ApoB) to track lipids in circulation in the fly.

Results and Conclusions:

The HPLC approach revealed a significant (Mann-Whitney U test $p<0.05$) accumulation of a unique triglyceride peak at steady state in flies with a gut microbiota when fed a high fat diet. Remarkably, most of the 65 unique lipid peaks were not altered on a high-fat diet nor between germ-free and colonized animals, indicating a robust ability of flies to regulate their lipidome. Post–prandially unique lipid peaks were observed in the early time points after feeding that are significantly reduced in later time points. I hypothesize that these peaks may represent circulating lipids in the fly, which are different from storage lipids. Moreover, the colonization status of animals also appears to influence their postprandial response, affecting both the distribution of lipid species and the levels of ApoLpp post-prandially. Further work will investigate the specific mechanism through which microbiota act, examining the effects of specific microbiota and fly genes on post-prandial lipid absorption and processing.

1453T **The role of Yolk protein 3 (YP3) in aging and development in *Drosophila melanogaster*** Natania Kurien, Krystal Maya-Maldonado, Nichole A Broderick Department of Biology, Johns Hopkins University

YP3 is one of three yolk proteins in *Drosophila* involved in vitellogenesis; it is used as an energy source during embryogenesis. This project aims to evaluate non-canonical functions of YP3 in other host physiological processes. We focused on the effect of YP3 on aging and development. Additionally, since *YP3* expression is induced by the microbiome, we also assessed the impact of YP3 on the microbiome. To do so, we used a YP3 loss of function mutant (ΔYP3) to evaluate development and longevity compared to its background control, W1118. We evaluated pupal development in conventionally reared (CR) flies, and our results showed that there was a moderate decrease in the total number of pupae in ΔYP3 flies compared to the W1118 strain, as well as an increased time to pupation. Interestingly, when we compared pupal development in germ-free flies (axenic) we observed no difference between the mutant and W1118 strains. Our results indicate that loss of YP3 negatively impacts pupal development, and this effect is possibly mediated by the microbiome. In looking into longevity, we observed an increased lifespan in ΔYP3 females as compared to W1118 females. No significant difference was seen in males. This work highlights non-canonical roles of YP3, specifically those in aging and pupal development. Further work with gnotobiotic flies re-associated with specific microbial communities will help expand upon interactions between YP3 and the microbiome.

1454T **Enteric pathogens modulate metabolic homeostasis in the *Drosophila melanogaster* host** Hoda Najjar Biomedical

Sciences, Qatar University

On quotidian basis, living beings work out an armistice with their microbial flora and a scuffle with invading pathogens to maintain a normal state of health. Although producing virulence factors and escaping the host's immune machinery are the paramount tools used by pathogens in their "arm race" against the host; here, we provide insight into another facet of pathogenic embitterment by presenting evidence of the ability of enteric pathogens to exhibit pathogenicity through modulating metabolic homeostasis in *Drosophila melanogaster.* We report that *Escherichia coli* and *Shigella sonnei* orally infected flies exhibit lipid droplet deprivation from the fat body, irregular accumulation of lipid droplets in the midgut, and significant elevation of systemic glucose and triglyceride levels. Our findings indicate that these detected metabolic alterations in infected flies could be attributed to differential regulation of peptide hormones known to be crucial for lipid metabolism and insulin signaling. Gaining a proper understanding of infection-induced alterations succours in curbing the pathogenesis of enteric diseases and sets the stage for promising therapeutic approaches to quarry infection-induced metabolic disorders.

1455T **Effect of multiple exogenous fecal exposures on Parkinson's-like symptoms in *Drosophila melanogaster*** Sharon Shaju[1], Ariella Tamariz[1], Regina Lamendella[1,2], Kathryn A. Jewett[1] [1]Department of Biology, Juniata College, [2]Contamination Source Identification (CSI)

Parkinson's disease (PD) is characterized mainly by shaking and stiffness, but also by sleep challenges, gut dysbiosis, increased protein aggregation, and a shortened lifespan. Disruptions in the *GBA* gene, encoding the enzyme glucocerebrosidase, predispose humans to PD. *Drosophila melanogaster* lacking this gene serve as a well-characterized PD model. Previous studies on PD in both humans and flies have shown differential abundance in the gut microbiome of affected and non-affected individuals. This motivates investigation of the potential effects of manipulating and influencing changes in the gut microbiome.

In these experiments, we used external fecal inoculation to mimic fecal transplantation. In preliminary testing, we applied a single dose of fecal matter. Western blotting showed a significant decrease in protein aggregation in the heads of PD-like flies fed food inoculated with healthy fecal matter, but no change in the bodies. 16S rRNA gene sequencing of the flies' dissected gut hints at Alphaproteobacteria being a biomarker for PD-like flies. This may indicate that non-PD fecal matter could be beneficial for improving PD symptoms.

We have started to explore multiple external inoculations to characterize the long-term effects of healthy and PD-like fecal exposure. At day 10, (4-5 exposures) we are analyzing the 16S rRNA, protein aggregation, and sleep, as well as measuring the effect of adult lifetime exposure on lifespan. Due to changes in the microbiome composition, we predict PD flies exposed to healthy fecal matter will display better outcomes, and potentially, non-PD flies exposed to PD fecal matter will begin to show PD-like symptoms.

1456T **A novel antidiuretic hormone governs tumor-induced renal dysfunction** Wenhao Xu Wuhan University

Maintenance of renal function and fluid transport is essential for both vertebrates and invertebrates to adapt to physiological and pathological challenges. It has been observed that subjects bearing malignant tumors frequently develop detrimental renal dysfunction and oliguria, with previous studies suggesting the involvement of chemotherapeutic toxicity and tumor-associated inflammation. However, the direct modulation of renal functions by tumors remains largely unclear. In this study, using conserved tumor models in *Drosophila,* we characterized isoform F of ion transport peptide (ITP_F) as the first fly antidiuretic hormone that is secreted by a subset of yki^{3SA}-gut tumor cells to impair renal function and cause severe abdomen bloating and fluid accumulation. Mechanistically, tumor-derived ITP_F targets the GPCR TkR99D in stellate cells (SCs) of Malpighian tubules (MTs), an excretory organ equivalent to renal tubules, to activate NOS/cGMP signaling and inhibit fluid excretion. We further uncovered previously unrecognizedantidiuretic functions of mammalian neurokinin 3 receptor (NK3R), the homolog of fly TkR99D, as pharmaceutical blockade of NK3R efficientlyalleviates renal tubular dysfunction in mice bearing different malignant tumors. Altogether, our results demonstrated a novel antidiuretic pathway mediating tumor-renal crosstalk across species and offered therapeutic opportunities for the treatment of cancer-associated renal dysfunction.

1457T ***drop-dead* mutants show early neuronal apoptosis and subsequent glial hyperactivation of innate immune signaling** Unmila Jhuti[1], Edward Blumenthal[2] [1]Biological Sciences, Marquette University, [2]Department of Biological Sciences, Marquette University

Hyperactivation of innate immunity (HII) has been linked to neurodegeneration (ND) both in humans and *Drosophila*. The *drop-dead* (*drd*) mutant fly is an excellent tool to study the relationship between HII and ND. *drop-dead* (*drd*) encodes a protein of unknown function whose absence causes early ND in adult fly brains. *drd* mutant brains start to show cell

death right after eclosion and the flies die within the first week of adult life. We have previously reported that several cell death pathways are activated in mutant brains in the final days before organismal death. However, the primary and earliest mechanism of cell death in mutants is apoptosis. Immunostaining against the apoptotic marker death caspase-1 (Dcp-1) is widespread as early as the day of eclosion, whereas HII is a late phenotype observed in older 4 day old mutant brains. Co-localization analysis following double immunostaining shows that Dcp-1 localizes with neuron-specific ELAV but not with glia-specific REPO. Along with apoptosis, older *drd* mutants also show HII. The trend of HII upregulation is age-dependent. qPCR showed that both 2 day old and 4 day old adult flies exhibit upregulation of different antimicrobial peptides (AMPs). *attacin A1, cecropin C,* and *diptericin B* showed approximately 10-20 fold upregulation in 2 day old mutant brains, whereas upregulation of the same genes in 4 day old mutant brains was 1000-3000 fold. qPCR showed 10 to 17,000 fold upregulated expression of 15 different AMPs in 4 day old *drd* mutants- *diptericin(A-B)*, *cecropin(A-C)*, *attacin(A-D), baramicinA, defensin, drosocin, drosomycin and metchnikowin.* Immunostaining and co-localization analysis with 2 AMP reporters (*AttA-GFP* and *DptA-GFP*) and glia-specific REPO suggest that the site of AMP overexpression is in glial cells. Our work shows that the absence of *drd* causes early neuronal apoptosis and late glial HII and suggests a more complex causal relationship between HII and cell death than has been reported in other *Drosophila* models of ND. Thus studying *drd* mutants can help in understanding the roles of both neuronal and glial cells and the timeline of activation of cell death and innate immunity pathways that lead to ND and organismal death.

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Keywords: apoptosis, hyperactivation of innate immunity, cell death, aging, neurodegeneration.

1458T **Neurofibromin-dependent modulation of metabolic homeostasis via neuronal and molecular mechanisms** Valentina Botero[1], Seth M. Tomchik[1,1,2] [1]Neuroscience and Pharmacology, University of Iowa, [2]Iowa Neuroscience Institute, University of Iowa

Neurofibromatosis type 1 (NF1) is a multisystemic, monogenetic disorder arising from *NF1* gene loss-of-function mutations. *NF1* encodes neurofibromin (Nf1), a large protein containing a central GAP-related domain (GRD). Neurofibromin modulates diverse cellular and molecular mechanisms. Individuals with NF1 present with a wide spectrum of clinical manifestations, including peripheral nerve-associated tumors, brain tumors, skeletal and vascular abnormalities, and neurocognitive and behavioral deficits. Evidence suggests that Nf1 regulates cellular and organismal metabolism in humans: individuals with NF1 have overall reduced stature, reduced body mass index, pituitary growth hormone deficiencies, muscle weakness, and increased insulin sensitivity. Despite indications of Nf1's involvement in metabolic regulation, the critical molecular mechanisms underlying metabolic effects remain largely unknown.

Leveraging the *Drosophila* NF1 model and its highly conserved signaling pathways, we investigated the Nf1-metabolism connection. *In vivo* genetic analysis revealed that the loss of Nf1 disrupts metabolic homeostasis, resulting in increased metabolic and feeding rates, altered lipid dynamics, and increased susceptibility to starvation. These metabolic effects mapped to a distinct set of interneurons in the nervous system, which can be dynamically modulated to increase metabolic rate. Further investigation revealed that Nf1 regulates metabolic rate via neuronal mechanisms, with additional contributions from muscle cells.

Neurofibromin's Ras GAP activity is mediated by its central GRD. We found that Ras signaling is critical for mediating Nf1 effects on metabolism and further explored its downstream signaling through genetic approaches. Expression of a constitutively-active ERK (ERK^{SEM}) partially phenocopied the metabolic phenotype observed with Nf1 loss. Additionally, *in vivo* genetic experiments revealed the necessity of the Raf/MEK/ERK and PI3K/AKT/mTOR pathways, with Nf1 likely mediating metabolism via coordinated actions of both pathways. These data reveal a novel interaction between Nf1 and metabolism – identifying neural circuits and signaling mechanisms responsible for metabolic regulation by Nf1.

1459T **The impacts of increasing intestinal barrier function on protein aggregation in *Drosophila melanogaster*** Samantha Le, Latrell Fomby, Anna Salazar Christopher Newport University

The impact of gut health on aging and disease has become an emerging field of science. Intestinal barrier function is associated with changes in health and aging, with gut integrity linked to lifespan in numerous organisms, including humans. Recent studies have utilized *Drosophila melanogaster* as a model organism to study the effects of intestinal barrier permeability on several markers of aging. Snakeskin (Ssk) is expressed between adjacent gut epithelial cells and is a septate-specific occluding junction protein that ensures the integrity of the gut by maintaining the barrier between the intestines and the rest of the body. These junctional proteins between intestinal epithelial cells play a major role in controlling the ability of bacteria, and other inflammation-inducing molecules, to pass from inside the gut to outside the gut, thereby limiting microbial dysbiosis and preventing the 'leaky gut' phenotype. These investigations utilize the GeneSwitch Gal4/UAS system in order to

overexpress Ssk in *Drosophila*, in a tissue-specific manner, in order to examine its effects on markers of aging, such as protein aggregation and mitochondrial dysfunction. This research aims to investigate whether increasing intestinal barrier function can be a possible treatment for diseases associated with aging in humans.

1460T **Endogenous Retroviruses and TDP-43 Proteinopathy Form a Sustaining Feedback to Drive the Intercellular Spread of Neurodegeneration** Yung-Heng Chang[1], Josh Dubnau[1,2] [1]Department of Anesthesiology, Stony Brook School of Medicine, [2]Department of Neurobiology and Behavior, Stony Brook University

Neurodegenerative diseases initiate focally and spread through neural circuits over time. Most amyotrophic lateral sclerosis and about half of frontotemporal dementia cases are associated with abnormal hyperphosphorylated cytoplasmic inclusions of TAR DNA Binding protein 43 (TDP-43). TDP-43 proteinopathy has been proposed to spread between cells via transfer of aggregated protein that seeds re-initiation of pathology by a prion-like templating mechanism. While there is evidence for intercellular movement of TDP-43 protein, this mechanism has not been shown to be necessary or sufficient to propagate pathology of the endogenous protein through neural tissue or to underlie cellular degeneration of recipient cells. We have used a fly in vivo model to investigate cell-autonomous and non-autonomous effects of TDP-43 aggregation. We will show both recently published and unpublished findings demonstrating feedback amplification between TDP-43 aggregation and endogenous retrovirus (ERV) expression. Inter-cellular transmission of ERVs underlies propagation of disease phenotypes from cell-to-cell.

We utilized a “humanized” *Drosophila* model in which the endogenous fly TDP-43 ortholog is replaced with physiological levels of the human TDP-43 gene. This fully rescues loss of the fly gene, yielding no evidence of neurodegeneration. We initiated TDP-43 proteinopathy focally in adult surface glia, and observed the impact on neurons and glia that express physiological levels and normal localization of the human protein. We demonstrate that such focal onset of TDP-43 proteinopathy triggers TDP-43 proteinopathy in neurons at a distance from the initiating source. We detect DNA damage foci in neurons that are adjacent to the glial source, and at further distances over time. This is associated with neuronal loss and shortened lifespan. We observe no evidence that this involves movement of TDP-43 protein. Instead, we demonstrate that all of the intercellular effects require expression of the mdg4-(gypsy) ERV within the glia where we initiate TDP-43 proteinopathy. Expression of this ERV within glia triggers TDP-43 pathology in nearby neurons. Expression of either *Drosophila* mdg4-ERV or of the human ERV, HERV-K, also are sufficient to initiate TDP-43 pathology in cell culture. We demonstrate that intercellular viral ERV transmission is sufficient to initiate TDP-43 proteinopathy in the recipient cells, and anti-retroviral drugs are capable of reversing TDP-43 aggregation. These findings suggest that TDP-43 proteinopathy and ERV expression are mutually reinforcing. Intercellular viral transmission may underlie propagation TDP-43 aggregation through the nervous system.

1461T **Developmental Alcohol Exposure in Alzheimer’s Disease Models of *Drosophila melanogaster*** Desiree Filardo Biological Sciences, San Jose State University

Fetal Alcohol Spectrum Disorder (FASD) results from developmental exposure to alcohol in mammals. It is the leading non-genetic cause of intellectual disabilities worldwide, and causes a variety of other developmental deficits, including developmental delays, low body weight, behavioral changes, and reduced brain size. We have established a model for developmental alcohol exposure (DAE) in *Drosophila melanogaster* and find that many of the phenotypes seen in other animal models of FASD can be replicated in flies. To identify molecular targets of DAE, we carried out a large-scale genetic screen for mutations that alter the sensitivity of flies to developmental alcohol exposure. From this screen, we identified the gene Dementin (*Dmtn*), which encodes the Drosophila ortholog of the Alzheimer Disease associated protein TMCC2. TMCC2 is required for normal processing of amyloid precursor protein. Mutations in *Dmtn* cause sensitivity to DAE, and DAE alters *Dmtn* expression. Finally, adult flies, after developmental exposure to alcohol, show climbing defects indicative of central nervous system dysfunction. Similar climbing defects are also seen in fly models of neurodegenerative diseases, including Alzheimer’s Disease. These results led us to hypothesize that DAE interacts with pathways that, when mutated, lead to neurodegeneration. To test this hypothesis, we have been examining the effects of DAE in mutations that serve as fly models of neurogenerative diseases, specifically Alzheimer’s Disease. Here we will present the results of experiments testing the interaction between DAE and mutations in the genes tau, presenilin, and amyloid-beta precursor protein (APP). Mutations in tau, presenilin, and APP serve as fly models for Alzheimer’s Disease-related neurodegeneration. Our preliminary data indicate that tau mutations render flies sensitive to the developmental effects of ethanol. We will present both survival and behavioral data to examine the interaction between ethanol and Alzheimer’s Disease in flies.

1462T **Investigating the impacts of intestinal Snakeskin knockdown on protein aggregation in *Drosophila*** CeOnna Battle, Connor Auby, Tyler Hyun, Anna Salazar Christopher Newport University

Many neurodegenerative diseases, such as Parkinson’s Disease and Alzheimer’s Disease, increase with age and are associated

with elevated protein aggregate levels. Previous research in fruit flies has discovered a relationship between increasing age and a deterioration in gut health, with developing intestinal barrier dysfunction tightly linked to mortality. Because of this tight association between organismal health and gut function, several markers of aging have been investigated in order to determine the role of barrier integrity on aging phenotypes. The primary focus of this study is to investigate a specific aging phenotype associated with several diseases of aging, whether intestinal barrier deterioration can impact protein aggregation. This research utilizes a knockdown in the protein Snakeskin (Ssk) in the midgut of *Drosophila melanogaster*, which induces intestinal barrier permeability. Ssk is a septate junction protein, equivalent to mammalian tight junctions, important for maintaining the integrity of the connections between adjacent gut epithelial cells. Ssk mislocalizes in aging flies, perturbing these junctions and causing intestinal permeability. Knocking down its expression in the gut, using the GeneSwitch Gal4/UAS system, causes a decrease in barrier function and a severely reduced lifespan, in addition to other markers of aging such as dysbiosis and inflammation. These experiments are investigating protein aggregation in the Ssk knockdown model in order to investigate whether unhealthy or aging guts exhibiting barrier dysfunction may be associated with neurodegenerative diseases linked with protein aggregation. Ideally, these insights will fuel a focus on intestinal health in the studies of several diseases with accompanying protein aggregation.

1463T **Ptth regulates lifespan through temporal and spatial activation of STING/NF-κB signaling during *Drosophila* metamorphosis** Ping Kang[1], Peiduo Liu[1], Michael B O'Connor[2], Hua Bai[1] [1]Genetics, Development and Cell Biology, Iowa State University, [2]Department of Genetics, Cell Biology and Development, University of Minnesota

The prothoracicotropic hormone (Ptth) is well-known for its role in controlling insect developmental timing and body size by promoting the biosynthesis and release of ecdysone. However, the role of Ptth in adult physiology is largely unexplored. Here, we show that Ptth null mutants (both males and females) are long-lived and increase resistance to oxidative stress. Transcriptomic analysis reveals that age-dependent upregulation of the innate immunity pathway is attenuated by Ptth mutants. Intriguingly, we find that Ptth regulates the innate immunity pathway specifically in fly oenocytes, the homology of mammalian hepatocytes. We further show that oenocyte-specific knockdown of NF-κB/Relish extends lifespan, while oenocyte-specific overexpression of NF-κB/Relish attenuates the lifespan extension of Ptth mutant. In addition to the oenocyte-specific regulation of the innate immunity pathway, we find that Ptth mutants exhibit reduced STING and NF-κB/Relish signaling during pupal development. Surprisingly, knockdown of either STING or NF-κB/Relish in pupal oenocytes significantly prolongs lifespan of adult flies. Thus, our findings reveal an unexpected longevity regulation mediated by Ptth hormonal factor and STING/NF-κB signaling in developing hepatocytes.

1464T **Intestinal serotonin promotes immunity and immune-related metabolism** Xiang Ding, Paula Watnick Division of Infectious Diseases, Boston Children›s Hospital, Harvard Medical School

Vibrio cholerae quorum sensing regulates the host survival during intestinal colonization in a *Drosophila melanogaster* oral infection model. We previously showed that the high cell density quorum-sensing regulator HapR represses pathogen anabolic metabolism and tryptophan uptake to activate host intestinal serotonin synthesis and innate immune signaling via an unknown pathway. Here, we show that, in uninfected flies, tryptophan is converted to serotonin in enterocytes. Serotonin released from these enterocytes activates receptors in enteroendocrine cells (EECs) of anterior midgut. Protein kinase C acts downstream of the serotonin receptor to activate ecdysone signaling, which promotes the transcription of *PGRP-LC*, signaling through the IMD pathway, and transcription of the enteroendocrine peptide Tachykinin, a repressor of lipid synthesis, and anti-microbial peptides (AMPs). Thus, we show that tryptophan, a microbe-impacted intestinal metabolite, regulates the ecdysone signaling and the intestinal innate immune response via serotonin.

1465T **A large-scale *in vivo* screen to investigate the roles of human genes in *Drosophila melanogaster*** Ashley Avila[1], Lily Paculis[1], Roxana Gonzalez Tascon[1], Belen Ramos[1], Dongyu Jia[2] [1]Georgia Southern University, [2]Kennesaw State University

Understanding the signaling pathways in which genes participate is essential for discovering the etiology of diseases in humans. The model organism, *Drosophila melanogaster*, has been crucial in understanding the signaling pathways in humans, given the evolutionary conservation of a significant number of genes between the two species. Genetic screens using *Drosophila* are a useful way of testing large quantities of genes to study their function and roles within signaling pathways. We conducted a large-scale genetic screen to identify which human genes cause an alteration in the morphology of the *Drosophila* eye. The *GMR-Gal4* was employed to activate a single *UAS*-human gene within each individual fly. In total, 802 *UAS*-human stocks corresponding to 787 human protein-coding genes underwent screening. Among these, changes in eye phenotypic characteristics such as texture, size, shape, bristles, and ommatidia were observed in 64 genes. Upon completion of the screen, bioinformatic databases were used to acquire biological information about the 64 genes such as up-regulated gene functions, cancer associations, and protein product homology between *Drosophila* and humans.

1466T **Interplay between intestinal barrier function, aging, and neurodegeneration** David Grace, Kayla Pauley, Stuart Saba,

Anna Salazar Christopher Newport University

The world population is aging, with the number of people over 65 more than doubling to 1.57 billion by 2050, making a concomitant elevation in numerous age-related pathologies, including neurodegenerative diseases such as Alzheimer's Disease, highly likely. Because of this, a clearer understanding of the pathophysiological changes accompanying aging, and the discovery of novel therapeutics to assist in aging phenotypes, are absolutely essential. Aging is a process marked by a continuous decline in multiple physiological functions, including the intestinal barrier function, which is tightly linked to longevity in *Drosophila melanogaster* and other organisms. We have previously shown that altered expression of occluding junctions in the guts of fruit flies can lead to various hallmarks of aging, including modulation of intestinal homeostasis, variations in microbial dynamics, changes in immune activity, and alterations in lifespan. Loss of a specific occluding junction, Snakeskin (Ssk), leads to rapid and reversible intestinal barrier dysfunction, altered gut morphology, dysbiosis, and a dramatically reduced lifespan. Remarkably, restoration of Ssk expression in flies showing intestinal barrier dysfunction rescues each of these phenotypes previously linked to aging. Intestinal up-regulation of Ssk protects against microbial translocation following oral infection with pathogenic bacteria. Furthermore, intestinal up-regulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends lifespan. Additionally, perturbing barrier function in the gut has non-cell-autonomous impacts, including alterations in the brain and muscle. These investigations add more information about the impact of the gut on tissue outside the gut and begin to address communication between the gut and the brain and muscles in disease models. These findings indicate that intestinal occluding junctions may represent prolongevity targets in mammals, in addition to their possible roles in intestinal dysfunction, aging, and disease. Current work utilizes cellular and molecular biological methodologies to build upon current knowledge to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, protein aggregation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan.

1467T **Establish a *Drosophila* model to investigate Epidermolysis Bullosa** Yan-Yan Lin[1], Anna C.-C. Jang[1], Yu-Chiuan Chang[2], Chao Kai Hsu[3] [1]Department of Biotechnology and Bioindustry Sciences, National Cheng Kung University, [2]Institute of Biomedical Sciences, National Sun Yat-sen University, [3]Department of Dermatology, National Cheng Kung University Hospital

Epidermolysis Bullosa (EB) is a rare genetic disorder characterized by an extreme fragility of the patients' skin. Currently, there is no cure for individuals afflicted by this condition, and despite the identification of common mutations responsible for EB, the underlying pathogenic mechanism has not yet been fully understood. In this study, by expressing the pathogenic variants found in patients with epidermolysis bullosa simplex, Dowling-Meara type (EBS-DM) in *Drosophila*, we establish an EBS-DM disease model which shows disrupted epidermal phenotype corresponding to the patients. This disease model not only serves as a tool for further exploring the pathogenic mechanism of EB but also provides a platform for expediting the screening of potential therapeutic drugs.

1468T **Building a Prostate Cancer Model: Aging Reduces the Nuclear Size of Virgin Flies and Glycolysis Levels of Mated Flies in *Drosophila melanogaster* Secondary Cells** Mischa Emery[1], Christian Massino[2], Susanne Broschk[2], Daimark Bennett[3], Klaus Reinhardt[2], Marko Brankatschk[2], Mirre Simons[4], Clive Wilson[5], Stuart Wigby[1] [1]Evolution, Ecology and Behaviour, University of Liverpool, [2]Technische Universität Dresden, [3]University of Manchester, [4]University of Sheffield, [5]University of Oxford

Introduction: Prostate cancer (PCa) is the most prevalent cancer of aging men, with a 1/3 of cases in the UK affecting those over 75. PCa cells differ from healthy prostate cells; they have elevated levels of glycolysis and cell division. The accessory gland of male *Drosophila melanogaster* contains secondary cells (SC) that share many characteristics with the human prostate. SC, unlike PCa cells, do not divide but instead, their nuclei grow in early adult life, with endoreplication occurring only in mated flies. The metabolic state of SC in young or aged flies is currently unknown. Based on previous work, we expected the nuclear size to increase with age, with accelerated growth in mated flies. In addition, we expected higher levels of glycolysis in the SC of flies allowed to mate, a phenotype that would be useful in a PCa model.

Methods: We housed male *D. melanogaster* as virgins, or in mixed-sex groups in which flies were allowed to mate freely ("mated"). Initial nuclear size experiment used 1 and 3 day old virgin males and aging experiments used 1, 3 and 5 week old virgin and mated flies. Flies were imaged with immunohistochemistry for nuclear size and fluorescence-lifetime imaging microscopy for metabolic status.

Results: We found, as previously reported, that in early adult life (day 1 to 3 following eclosion), there is a significant increase in SC nuclear size. However, at weeks 1 and 3 there are no differences in nuclear size between virgin and mated flies, while at week 5 there is a significant reduction nuclear size of virgin flies. At weeks 1 and 3 glycolysis is higher in mated flies however, at week 5 there is no difference in glycolysis level between virgin and mated flies.

Conclusions: We investigated SC cell characteristics that are important for building a PCa model. Flies aged to 5 weeks

did not exhibit nuclear size changes nor glycolysis levels that would mimic a PCa cell phenotype. Further tests can show if endoreplication is occurring in the older flies, which may reveal DNA replication without an increase in nuclear size. Further work is required to establish whether aged flies have a phenotype that mirrors normal, aged prostate cells rather than PCa cells. This study provides a background for the application of age-extending treatments such as pharmaceuticals or dietary changes to further characterise SC as a PCa model.

1469T **Nazo, the *Drosophila* homolog of the NBIA-mutated protein – c19orf12, is required for triglyceride homeostasis in *Drosophila* Gut** Sreejith Perinthottathil, Rajnish Bharadwaj Pathology and Lab Medicine, University of Rochester Medical Center

Lipid dyshomeostasis has been implicated in a variety of diseases ranging from obesity to neurodegenerative disorders such as NBIA. Here, we uncover the physiological role of Nazo, the *Drosophila* homolog of the NBIA-mutated protein – c19orf12, whose function has been elusive. Ablation of *Drosophila* c19orf12 homologs leads to dysregulation of multiple lipid metabolism genes. *nazo* mutants exhibit markedly reduced gut lipid droplet and whole-body triglyceride contents. Consequently, they are sensitive to starvation and oxidative stress. Nazo localizes to ER-lipid droplet contact sites and is required for maintaining normal levels of Perilipin2, an inhibitor of the lipase – Brummer. Concurrent knockdown of Brummer or overexpression of Perilipin2 rescues the *nazo* phenotype, suggesting that this defect, at least in part, may arise from diminished Perilipin2 on lipid droplets leading to aberrant Brummer-mediated lipolysis. Our findings provide novel insights into the role of c19orf12 as a possible link between lipid dyshomeostasis and neurodegeneration, particularly in the context of NBIA.

1470T **Dihydromotuporamine C acts through Rho1 and diaphanous to inhibit cell migration.** Laurie von Kalm, Corey Seavey Biology, University of Central Florida

The motuporamines are a promising class of anti-metastatic compounds. In mammals, Dihydromotuporamine C (Motu33) has been shown to activate the small GTPase RhoA, however, little is known about subsequent downstream events leading to cell migration inhibition. In the present study, we investigated the mechanism of action of Motu33 and a synthetic derivative, Motu-(CH_2)-33, in Drosophila by manipulating the gene dose of positive and negative regulators of actin dynamics. Consistent with previous findings, reduced gene dose of Rho1 (the Drosophila RhoA ortholog) attenuates motuporamine activity, confirming that RhoA/Rho1 is targeted by these compounds. Actin-myosin contraction is controlled by the Rho1-ROCK-myosin regulatory light chain (MRLC) pathway. Reduced gene dose of the myosin binding subunit of myosin phosphatase, a negative regulator of the Rho1-ROCK-MRLC pathway, enhances motuporamine activity indicating that the motuporamines stimulate actin-myosin contraction through activation of the myosin regulatory light chain. Rho1 also activates diaphanous (dia) to control actin polymerization. Surprisingly, reduced gene dose of dia enhances motuporamine activity, suggesting that the motuporamines act on dia in a Rho1-independent manner. Reduction in gene dose of the Drosophila Rac orthologs also enhances motuporamine activity. In contrast, motuporamine activity is unaltered by reduced gene dose of slingshot (ssh) which acts to trigger actin severing and depolymerization. Since ssh is directly regulated by Rac, the enhanced activity of motuporamines observed when Rac gene dose is reduced may reflect an indirect mode of action on the Rac GTPases leading to increased Rho1 activity. In summary, these findings demonstrate that motuporamines act through RhoA/Rho1 and diaphanous to regulate actin-myosin contractility and actin polymerization.

1471T **Characterization of seizure susceptibility in a *Drosophila* model of KDM5C-associated X-linked intellectual disability disorder** Bethany K Terry[1], Matanel Yheskel[1], Hayden AM Hatch[1,2], Julie Secombe[1,2] [1]Department of Genetics, Albert Einstein College of Medicine, [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine

KDM5C-associated X-linked intellectual disability disorder (XLIDD), also known as Claes-Jensen syndrome, is a rare neurodevelopmental disorder caused by variants in the Lysine (K)-specific demethylase 5C (*KDM5C*) gene. In addition to physical growth challenges, individuals with KDM5C-associated XLIDD have been reported display a spectrum of different neurological changes, which include intellectual disability, gross and fine motor delays, emotional and behavioral changes, and epilepsy. However, although seizures have been reported in up to a third of individuals with this disorder, how alterations in KDM5C activity result in the promotion of seizures is not well understood.

Previous work in our lab has demonstrated that perturbation of KDM5, *Drosophila*'s KDM5C ortholog, can impact neurodevelopment and cognitive functioning. In this study, we utilized our *Drosophila* models to investigate the role of KDM5 in seizure susceptibility. To do this, groups of adult flies were exposed to heat or mechanical stress and then recorded for several minutes. Video recordings were then assessed to determine the number of flies that seized and the time that each fly seized for. Interestingly, we found that expression of patient-related variants of *Kdm5* increases the proportion of flies that seize in response to mechanical stress in comparison to control animals. Furthermore, we found increased seizure

susceptibility and duration following neuron-specific knockdown of *Kdm5* in comparison to controls. Having established the increased propensity of our *Drosophila* model to seize, ongoing work will aim to identify the mechanisms underlying the seizures that occur following KDM5 perturbation.

1472T **A *Drosophila* model for infection-induced loss of intestinal barrier function and homeostasis** Madison Clark[1], Nichole Broderick[2] [1]Johns Hopkins University, [2]Biology, Johns Hopkins University

Gastrointestinal diseases, such as inflammatory bowel disease and antibiotic-induced colitis are major concerns worldwide. While the underlying causes of gut disease are not completely understood, innate immune responses and a healthy gut microbiome have been suggested as key to the maintenance of intestinal homeostasis. *Drosophila* is an ideal host to study host-microbe interactions as it has a low complexity microbiome that is easily manipulated to study the contributions of individual microbes on gut health. This study characterizes the response of *D. melanogaster* to a novel pathogen, *Chromobacterium subtsugae,* a Gram-negative environmental bacterium known for its quorum sensing production of the purple-pigment violacein. We fed *C. subtsugae* to adult *D. melanogaster* and found flies are significantly more susceptible to a purple pigmented strain that over produces violacein (ΔvioS), compared to the wild-type non-pigmented strain. Initially after infection, pathogen load is high, but gradually decreases as the infection progresses. By day 4, almost no pathogen can be detected in flies infected with either strain. Interestingly, most death post infection occurs 7-10 days after ingestion. This suggests flies can clear the pathogen early in the course of the infection and that constant association of the pathogen with the host is not necessary for death. In examining the early events in the course of infection that contribute to death, we found that ingestion of the ΔvioS strain disrupted barrier integrity, leading to gut leakiness. Initially, immune and homeostatic mechanisms to repair intestinal damage are induced. However, around day 6 post-ingestion, we observed that total gut length was reduced by about 30% and this was associated with an increase in undifferentiated cells in the gut. We will discuss our exploration of signaling pathways contributing to this response as well as the role of the microbiome to host survival. We propose that the *D. melanogaster-C. substugae* infection model provides a useful new tool to study the dynamics of gut disease that lead to the loss of whole-organism homeostasis, such as in mammalian sepsis. Understanding how microbes interact with a host to cause such diseases will allow us to identify innovative mechanisms to limit the damage microbes cause during infection.

1473T **Trigger warning: Do psychological stressors trigger neurodegeneration in a *Drosophila* TDP-43 model?** Narmin H Mekawy[1,2], Swetha BM Gowda[3], Joshua Dubnau[1,2,3] [1]Department of Neurobiology and Behavior, Stony Brook University, [2]Program in Neuroscience, Stony Brook University, [3]Department of Anesthesiology, Stony Brook Medicine

Aging is a major risk factor of onset of neurodegeneration, however the rate of progression is not impacted by the age of onset. Other risk factors include post-traumatic stress disorder, emotional stress, obesity, and environmental toxins. It is not clear whether and to what extent biological aging, cumulative effects of lifelong stress, or early life stresses act to trigger onset of neurodegeneration. The mechanisms by which psychological stress may drive disease are also not known. Genetically inducing TDP-43 pathology animal models is sufficient to trigger progression of neurodegenerative phenotypes. For example, in *Drosophila*, inducing TDP-43 over-expression in glial cells is known to trigger spread of neurodegeneration to other glia and to neurons in. This is also associated with loss of nuclear TDP-43 and accumulation of cytoplasmic inclusions. To investigate the upstream triggers of neurodegeneration, we are testing the effects of “psychological stressors” by providing stressful perceptual experiences to the flies that have or are genetically sensitized towards TDP-43 neurodegeneration. We are testing the impacts of these manipulations on TDP-43 protein pathology and effects on neurodegenerative phenotypes as well as on lifespan. In this poster, we will describe the experimental strategies used to test the impacts of perception of predation risk, frustration of mating potential and restraint stress.

1474T **Gene-by-Environment Analysis of Sleep Deprivation on Diet Choice in *Drosophila*** Jhilam Dasgupta, Gabbie Bicanovsky, Miled Maisonet-Nieves Biological Sciences, University of Alabama

More than 30% of individuals in the United States suffer from metabolic syndrome, which is a risk factor for diseases including cardiovascular disease, diabetes, and hypertension. Disruptions to sleep can increase risk of obesity in humans. *Drosophila melanogaster* is an established model organism that can be used to illustrate the effects of sleep deprivation on metabolism. Sleep varies between individuals, and it is important to understand how genetics impact metabolic health across wild type lines with differing sleep phenotypes. To study these effects, *Drosophila Genetics Reference Panel* (DGRP) short-sleeping and long-sleeping strains were placed in a *Drosophila* sleep deprivation apparatus, which shakes the flies for five seconds in one-minute intervals over a three-day period. Fly activity was monitored to compare daytime and nighttime activity between short-sleeping and long-sleeping DGRP strains. Following the sleep deprivation period, a choice capillary feeding (CaFe) assay was performed to measure consumption of high sugar (30%) sucrose, high fat (coconut oil derived ketogenic mix) and normal diet (10% sucrose). We have chosen these diets to better represent the most common westernized diets that are correlated with reduced metabolic health. This experiment seeks to identify the extent to which sleep deprivation affects dietary choice

in sleep mutant strains. In the future, by replicating these experiments across multiple DGRP lines, we will be able to identify gene polymorphisms that may increase or decrease diabetic risk factors due to sleep deprivation. Due to conservation of gene function between *Drosophila* and humans, results will likely be useful in determining the relationship between sleep and metabolic health in humans.

1475T **Rescue effects of the antimicrobial peptide LL-37 on beta amyloid-induced pathology in a Drosophila model of Alzheimer's disease** MaiLan Kasch[1], Belal Alatasi[1], Kenneth Owyang[1], Julia Aguiar[1], Alison Swick[1], Miles Maybrun[1], Kayla Azad[1], Waleed Alsibai[1], Morgan Wong[1], Annelise Barron[2], Jeremy Lee[1] [1]Department of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA 95064, UC Santa Cruz, [2]Department of Bioengineering, Stanford University School of Medicine, Stanford, CA 94305, Stanford University

Alzheimer's disease (AD) is characterized by the aggregation of neurotoxic amyloid plaques, largely composed of the peptide amyloid beta (Aβ). Research has shown that when bound in vitro with Aβ, the human antimicrobial peptide LL-37 decreases amyloid aggregation (De Lorenzi, et al., 2017). Based on these findings, we hypothesized that the expression of LL-37 ameliorates the effects of Aβ's neurotoxicity. To test this hypothesis in vivo, we generated four transgenic fly lines that pan-neuronally express the human peptides LL-37 and Aβ individually or together and compared these genotypes with non-expressing controls.

When expressed alone, Aβ had detrimental effects on fly lifespan and survivorship, but co-expression with LL-37 resulted in a significant rescue of adult lifespan and full rescue of survivorship to adult. This suggests that co-expression of LL-37 with Aβ partially alleviates the effects of Aβ. We then used RNA sequencing to examine the gene expression profiles of the different genotypes. We discovered that the largest category of genes that were upregulated in Aβ-expressing flies were genes encoding proteins involved in proteolysis. We also found that in flies co-expressing LL-37 with Aβ, many of these proteolytic genes were subsequently downregulated in comparison to Aβ-expressing flies. Similarly, proteolytic genes that were downregulated in Aβ-expressing flies as compared to controls were subsequently upregulated when LL-37 was coexpressed with Aβ. This evidence indicates that the proteolytic genes that were misregulated in Aβ-expressing flies were at least partially rescued by expression of LL-37. Additionally, there were no misregulated genes for which misexpression was exacerbated by co-expression of LL-37, which suggests that the attenuation of the misregulation is not random.

To further investigate the mechanisms of LL-37's effects on AD-like pathology, we are performing rapid iterative negative geotaxis and olfactory learning assays to investigate the neurodegenerative effects of Aβ with and without expression of LL-37 on motor function and memory. We are also exploring the phenotypic effects of Aβ through expression with a GMR-Gal4 driver, which limits expression to the eyes. The results from these experiments could further shed light on whether LL-37, and/or factors that increase its expression, could serve as promising targets for the future development of Alzheimer's disease therapeutics.

1476T **Illuminating the non-genetic factors of immune activation** Yu Yang Boston University

Immune activation is a tightly controlled process, as over-activation leads to autoimmunity, while under-activation lets bacteria proliferate unchecked. However, even genetically identical *D. melanogaster* infected with the same number of bacteria will die at different time points. Since many non-genetic factors including sex, age, past infections, and the environment can change immune response, two flies with similar life histories can have different immune recognition speeds and therefore a different pathogen growth trajectory. A fly's ability to survive an infection depends on rapid immune activation controlling the bacterial load in the first few hours of infection. However, the exact interplay between bacteria growth and immune response in individual animals has yet to be observed. By observing and modeling the individual variation in immune response, we can discover new targets that control immune activation. I will approach this question using bioluminescent bacteria, allowing me to continuously track infection progress in individual flies in a high throughput manner. To make the bacteria, I transform them with the iLUX plasmid, which generates bioluminescence without the need for any additional substrate. The signal is closely correlated with bacterial load in the fly. I will use both gram-positive and gram-negative bacteria (*E. faecalis, E. coli, and S. marcescens*) to probe the activation speed and variability of the two major innate immune pathways: Toll and IMD. At the same time, I will track fly immune response using flies that produce mCherry when producing antimicrobial peptides (AMP) such as diptericin and drosomycin. I will image the Dpt-mCherry and Drs-mCherry enhancer reporter flies injected with bioluminescent bacteria in a microplate reader for two days after injection, tracking how the iLUX signal changes in response to immune activation. Using these data, I will create a computational model of resistance and death. From this, we can study the random variations between individuals and model non-genetic drivers of resistance and tolerance.

1477T **The impact of timing on the protective effect of chronic infection in *Drosophila melanogaster*** Grace Ginder, Moria C. Chambers Biology, Bucknell University

One potentially influential category of microbes are ones that initially cause an acute infection and persist as a chronic infection if they don't kill their host. These chronic infections can influence many elements of host biology including metabolism, behavior, and immunity to future infection. Our lab has found that in the fruit fly, *Drosophila melanogaster*, chronic bacterial infection confers non-specific protection against more lethal bacterial infections given a week later. However, it is unclear how time between initial infection and secondary infection impacts this protective effect. We hypothesized that there is an optimal window for protection, as longer times between infections may allow the host to recover from the first infection but may also result in lower immune gene expression as well. To test how the time between the two infections impacts protection, three commonly used fly lines – w1118, Canton S, and Oregon R – were injected with a sub-lethal dose of *Serratia marcescens* that causes chronic infection and promotes continued expression of antimicrobial peptide genes at seven days post-infection. Then, 3, 7, or 14 days later, the flies were injected again with a lethal infection of *Providencia rettgeri*. To assess overall protection, survival was tracked for 8 days post-infection. To specifically determine the impact of chronic infection on resistance, bacterial load was measured 12 hours post-infection. The protective effect was strongest in w1118 flies, which showed significant protection at all times tested. Similar to previous results in the lab, our bacterial load assays indicated that protection is likely due to changes in resistance. We are currently testing how the strength of protection correlates with both antimicrobial peptide gene expression and phagocytic index. To functionally test the role of phagocytes in this protection, in the future we will assess whether fly lines missing key phagocytic genes can be protected by chronic infection.

1478T **Identifying novel links between cardiovascular disease and insomnia by *Drosophila* modeling of genes from a pleiotropic GWAS locus** Farah Abou Daya[1], Torrey Mandigo[2], Lily Ober[1], Dev Patel[1], Matthew Maher[2], James Walker[2], Richa Saxena[2], Girish Melkani[1] [1]Pathology, University of Alabama at Birmingham, [2]Massachusetts General Hospital

Cardiovascular disease (CVD) is the leading cause of death worldwide. CVD is associated with insomnia such that insomnia symptoms double the risk of incident CVD. However, how they are related is unknown. Two recent genome-wide association studies (GWAS) identified genetic loci significantly associated with insomnia, including one locus with five nearby genes that are associated with CVD in an independent GWAS. Therefore, we hypothesized that genetic predisposition to insomnia contributes to the development of CVD. To test this and identify causal genes at this locus, we used Drosophila melanogaster, which are well- established model systems for sleep and cardiac studies. To assess the role of these genes on cardiac physiology, we used the cardiac-specific Hand-Gal4 driver to perform genetic knockdown (KD). One-week-old Drosophila progeny were then used for semi-intact microscopic heart preparation followed by high-speed videography to assess cardiac physiology. Similarly, to assess their role in sleep, we used the neuronal-specific Elav-Gal4 driver to perform KD of these genes. Sleep and locomotor activity of one-week-old flies were monitored using the Drosophila Activity Monitoring System. We found that neuronal and cardiac-specific RNAi KD of four genes conserved in Drosophila: Lsn, ATPSynC, Bruce, and Imp, contributes to compromised sleep and cardiac performance, respectively. Cardiac-specific KD of Lsn led to significant cardiac dilation and reduced fractional shortening, a measure of cardiac performance. KD of ATPSynC led to significantly reduced fractional shortening without dilations. Furthermore, Lsn and ATPSynC KD hearts had disrupted actin- containing myofibrillar organization. Suppression of Lsn increased Pericardin deposition, indicative of fibrosis. Also, the cardiac suppression of ATPSynC and Lsn significantly shortened lifespan. Neuronal-specific KD of three genes significantly disrupted sleep. Furthermore, KD of ATPSynC and Lsn in the heart disrupted sleep in a non-cell-autonomous manner. This work provides novel insights into genetic mechanisms linking CVD and insomnia, highlighting the potential importance of these four genes in the development of both diseases.

1479T **Hedgehog signaling converges with HAP40 to control intestinal aging and disease** Jennifer I Alexander[1,2], Alana M O'Reilly[3], Christopher J Lengner[1] [1]Biomedical Sciences, University of Pennsylvania, [2]Cancer Signaling and Microenvironment, Fox Chase Cancer Center, [3]Cancer Signaling and Micronenvironment, Fox Chase Cancer Center

Alarmingly, Colorectal Cancer (CRC) incidence is disproportionately rising in young patients (EAO-CRC) without known cause. However, the underlying mechanisms at the intersection of aging biology and EAO-CRC pathogenesis remain elusive. By leveraging the powerful molecular genetics of the fly-to-human intestinal organoid pipeline, our goal is to delineate the mechanisms of aging that drive EAO-CRC. We discovered a novel Hedgehog (Hh)-dependent mechanism that balances autophagy-based cellular repair and proliferative regeneration to sustain healthy tissue aging. Specifically, we identified the Hh effector Patched (Ptc) as the critical switch for regulating both processes. We also found that the autophagy-associated transcript, HAP40, was reduced in Ptc hypomorphs. We then determined the impact of gain or loss of HAP40 in intestinal cells and observed evidence of increased cellular aging coupled with decreased survival and proliferation. We then queried human-derived transcriptomic data to assess HAP40 status in Hh-dependent cancers and found that 7% of patients displayed either gene amplification or deep deletion. We next compared the diagnosis age of patients, finding that patients bearing mutations in the HAP40 gene were diagnosed younger than those without mutations. Moreover, survival probability of patients with

normal HAP40 and Hh pathway signaling was significantly better than patients with mutant HAP40 or Hh effectors. The phenotypic similarities and dependency of HAP40 expression on Ptc suggests a new link between Hh and HAP40 signaling that is critical for intestinal tissue maintenance and aging regulation in flies. Taken together, this suggests a model in which Hh pathway components intersect with an understudied gene as a dual signaling cassette that impacts patient outcomes. Our next goal is to generate isogenic patient-derived CRC organoids to validate our experimental and transcriptomic data. Delineation of the sequential activation steps of this novel pathway will establish a new paradigm for the biological impact of Hh signaling driving EAO-CRC.

1480T **A Drosophila model of a rare Congenital Disorder of Glycosylation associated with ALG10** Suraj R Math[1], Juan Miguel M Guerra Solano[1], Athena Q Ye[1], Brittany S Leger[1], Torrey R Mandigo[1], Christina M Woo[2], Richa Saxena[1], James A Walker[1] [1]Center for Genomic Medicine, Massachusetts General Hospital, [2]Department of Chemistry and Chemical Biology, Harvard University

Congenital disorders of glycosylation (CDGs) are a group of rare multifaceted genetic disorders resulting in severe development problems, neurological deficits, seizures, and metabolic issues. They affect the post-translational addition of *N*-glycans to proteins which can alter their cellular processes including solubility, stability, cellular localization, and molecular trafficking. The *N*-glycosylation pathway relies on the activity of transmembrane asparagine-linked glycosylation (ALG) enzymes functioning in the endoplasmic reticulum (ER). Most enzymes involved in the *N*-glycosylation pathway have an associated CDG; however, to date no CDG has been described for ALG10, which catalyzes the terminal step of lipid-linked oligosaccharide (LLO) biosynthesis. We have found that the human genome encodes two paralogs *ALG10* and *ALG10B* arising from a recent duplication in primate evolutionary history. We subsequently identified a human subject with homozygous variants in both *ALG10* and *ALG10B*, suffering from progressive myoclonic epilepsy. In contrast to humans, *Drosophila* possesses a single *Alg10* gene. Neuronal RNAi knockdown of Alg10 in flies led to bang-sensitive seizures indicating a CDG-like phenotype in flies. Glycoproteomic analysis of *Alg10* knockdown was used to identify key target genes of the *N*-glycosylation pathway in the brain. We are utilizing our fly model to screen potential therapeutics for ALG10-CDGs. We are also conducting a structure-function analysis using CRISPR-Cas9 to introduce mutations into specific highly conserved regions of *Alg10*, to identify key residues and domains for its catalytic activity, transmembrane domains and potential signal peptide sequences required for localization to the ER.

1481T **Metabolomics and lipidomics studies reveal altered metabolism in a *Drosophila melanogaster* disease model of *PLA2G6*-associated Neurodegenerative disease (PLAN)** Shahira Helal Arzoo[1], Rubaia Tasmin[2], Eliezer Heller[3], Jeremy Purow[3], Josefa Steinhauer[3], Surya Jyoti Banerjee[4] [1]BIOLOGY, Texas Tech University, [2]Biology, Texas Tech University, [3]Biology, Yeshiva University, [4]Biological Sciences, Texas Tech University

The recently described *Drosophila melanogaster* models of *PLA2G6*-associated neurodegeneration (PLAN), which carry loss of function mutations in the calcium-independent phospholipase A2 (iPLA$_2$) VIA gene, exhibit age dependent locomotor defects, reduced life-span, and female specific fertility defects. We previously showed that wild-type *iPLA$_2$-VIA-PB* localizes to mitochondria in female germ cells, and that in aged *iPLA$_2$-VIA* null mutant female flies, the germ cells show abnormal mitochondrial aggregation and elevated apoptosis. These observations, along with data from other systems suggesting important roles of *iPLA2-VIA* at mitochondria, led us to investigate whether systemic mitochondrial functions are perturbed in *iPLA2-VIA* null mutants. We hypothesized that age-dependent and sex-specific metabolic abnormalities would be observed in *iPLA2-VIA* mutant flies. Therefore, we performed mass-spectrometry of small metabolites and of lipids isolated from young (< 1 week) and aged (1 month) *iPLA2-VIA* null mutant and isogenic control, adult male and female flies, and we analyzed the data with SIMCA and MetaboAnalyst software. We identified 195 small metabolites and 379 lipids that passed the quality control. We identified the metabolites and lipids that are significantly up- or down-regulated by at least 1.5-fold in the aged control flies compared to the aged *iPLA2-VIA* null mutant flies. In the aged male mutant flies, 27 metabolites are upregulated and 55 metabolites are downregulated; furthermore, 3 lipids are upregulated and 303 lipids are downregulated. In the aged female mutant flies, 46 metabolites are upregulated and 36 metabolites are downregulated; further, 130 lipids are upregulated and 102 lipids are downregulated. The Principal Component Analysis (PCA) reveals that there is almost complete overlap of the metabolic profiles between young *iPLA2-VIA* null mutant and control male flies, and partial overlap of those in the aged mutant and control male flies. The PCA analysis of lipidomics data shows that the lipid profiles of only the young mutant and old control male flies are very different. Conversely, complete separation of the metabolites is observed in the young and aged mutant females, and in the young and aged control females. Interestingly, the profile of metabolites in the young *iPLA2-VIA* null mutant female flies has close similarity with that of the old control female flies, suggesting that *iPLA2-VIA* mutant females experience premature aging. This raises the possibility that the female fertility defect and associated ovarian phenotypes derive from the somatic metabolic environment, consistent with our data showing that *iPLA2-VIA* knockdown in somatic tissues only can phenocopy the ovary defects of the null mutant.

1482T **Toward identification of protective modifiers of perinatal lethality in genetically divergent mice with Cornelia de Lange Syndrome.** Catherine J. Brunton, Steven C Munger The Jackson Laboratory

Cornelia de Lange Syndrome (CdLS) is a rare multisystem malformation syndrome that presents at birth with a spectrum of deficits, including limb truncations, craniofacial defects, heart defects, developmental delays, and early lethality. Mutations in cohesin complex associated genes, predominantly in NIPBL, are implicated in most cases of CdLS, yet 30% of affected humans lack mutations in canonical genes. Furthermore, a wide range of disease manifestation from mild to severe cases suggests that other gene variants could play a protective or sensitizing role. While the low incidence of CdLS (1:10,000 live births) prohibits large genome-wide association studies in the human population, emerging genetic resources in mice hold promise for finding genetic modifiers of CdLS-associated phenotypes.

The common C57BL/6J (B6) laboratory strain is particularly sensitive to a hypomorphic gene trap allele of *Nipbl*, with few heterozygous mice surviving to one week of age. In contrast, F1 hybrid progeny from reciprocal crosses of B6 with the evolutionarily divergent CAST/EiJ (CAST) strain are largely protected from effects of the *Nipbl* gene trap allele, with most heterozygous CASTB6F1 progeny living to adulthood and maintaining fertility. These observations suggest that the CAST genetic background harbors at least one dominant, protective modifier allele.

To profile genomic differences between our two CdLS-affected mouse strains, we will perform allele-specific expression analysis on RNA-seq data obtained from developing cerebral cortex and kidney of female *Nipbl*+/- and *Nipbl*+/+ embryos harvested from both the CdLS-sensitive B6 and CdLS-protective CASTB6F1 strains. Within CASTB6F1 *Nipbl*+/- individuals, we will apply EMASE software to map genes that exhibit unequal transcript abundance between the CAST and B6 alleles (i.e., allelic imbalance). Transcript abundance estimates in CASTB6F1 *Nipbl*+/+ and B6-derived heterozygous and wild-type tissues will serve as controls. Cis-acting strain variation likely underlies allelic differences in gene expression, and one or more genes exhibiting differential allelic expression in tissues harvested from affected individuals may be associated with the observed strain differences in viability and other defining features of the CdLS phenotype. Ultimately, identification of protective allele(s) could provide a genetic marker for enhanced diagnosis or potentially a novel gene target for therapeutic interventions in CdLS patients.

1483T **Multiple genetic loci influence vaccine-induced protection against *Mycobacterium tuberculosis* in genetically diverse mice** Sherry Kurtz[1], Richard Baker[2], Daniel Gatti[3], Karen Elkins[4] [1]CBER/OVRR, US Food and Drug Administration, [2]University of Massachusetts, [3]Jackson Laboratories, [4]US Food and Drug Administration

Diversity Outbred (DO) mice are a genetically and phenotypically diverse small animal population uniquely suited for performing genetic mapping studies. We used DO mice to study vaccine-induced responses to *Mycobacterium tuberculosis* (Mtb) challenge. Over 1000 DO mice were vaccinated with BCG, challenged with Mtb 8 weeks later, then followed for 14 weeks before euthanasia and sample collection. Compared to naïve DO mice, BCG-vaccinated animals were largely protected from early morbidity, and across the population lung and spleen Mtb burdens were reduced in vaccinated mice. Some vaccinated mice contained high levels of *M.tb*. in lungs and spleens, while others apparently cleared *M.tb*. from spleens. Evaluation of lung tissues revealed diverse disease pathologies in terms of the proportion of inflamed lung tissue as well diverse pathologies. From a subset of 300 DO mice, levels of 37 cytokines were quantified in lung tissue samples collected necropsy. Cytokine expression patterns across the DO population were heterogenous, and several cytokines significantly correlated with lung or spleen bacterial burdens. Infection outcomes, such as lung and spleen Mtb CFU and cytokine data, were used to perform Quantitative Trait Locus (QTL) mapping. Mapping revealed novel QTL associated with disease traits such as weight loss and lung and spleen Mtb burdens. Similar to observations in humans, BCG vaccination provided greater protection in DO mice against systemic versus pulmonary Mtb, and QTL analyses revealed 2 loci associated with the ratio of lung/spleen Mtb. Genetic mapping also revealed several novel QTL associated with lung cytokines including lung IL1a, CXCL1 and IL13. These cytokine-associated loci are largely independent of infection trait QTL and may reveal novel regulatory pathways for these cytokines and novel associations with protection. Taken together, our analyses revealed novel genetic loci associated with vaccine-induced protection against Mtb and point to genes associated with these loci. Importantly, results imply that protection is multigenic, influencing various aspects of immunity that collectively are critical for protection.

1484T **Generation, Genetic Characterization and Phenotypic Analysis of a Novel *Serpina1* Rat Model** Brooke Bowman, Daniel Davis, Elizabeth Bryda University of Missouri

SERPINA1 is a gene that codes for the alpha-1 antitrypsin protein, a serine protease inhibitor produced primarily in the liver. Alpha-1 antitrypsin plays a key role in the inactivation of proteolytic enzymes in lung tissue, especially neutrophil elastase. Excess neutrophil elastase can cause lung damage. Mutations in *SERPINA1* can result in a genetic disorder known as alpha-1 antitrypsin deficiency. Over one hundred *SERPINA1* variants have been associated with this disorder which leads

to adverse health effects, particularly liver and lung disease. One variant is the S allele (p.Glu264Val). The S allele produces an atypical type of alpha-1 antitrypsin that results in reduced serum alpha-1 antitrypsin concentration. Inheritance of this allele is associated with a small risk for lung disease. Using CRISPR-Cas9 genome editing, we generated a novel rat model carrying the *Serpina1* S allele. This is the first reported rat model with a genetic alteration in the *Serpina1* gene. As part of the phenotypic characterization of the model, we have optimized salivary chloride assays to test the hypothesis that the *Serpina1* mutant rats have reduced chloride levels. This new rat line will provide an animal model for researchers interested in studying *Serpina1* and understanding the biological consequences of mutating the gene. (Funded by NIH grant P40 OD011062 to ECB).

1485T **Mitochondrial phenotypes in BXD models of aging and Alzheimer's disease** Mikhail Tiumentsev[1], Stephen E. Alway[2], Richard Cushing[2], Malik Hullette[2], Jesse Ingels[2], John T. Killmar[1], Melinda McCarty[2], Hector G. Paez[2], David Ashbrook[1] [1]Genetics, Genomics and Informatics, The University of Tennessee Health Science Center, [2]The University of Tennessee Health Science Center

The connection between mitochondrial dysfunction and aging is well-established, and mitochondrial dysfunction seems to contribute to the pathogenesis of Alzheimer's disease (AD). However, the exact mechanistic nature of this connection is not fully understood. One of the main scientific gaps is that the interactions between mitochondrial function, multiple genetic variants, and health outcomes are not known. Many animal models use a single genome in a single environment to address this problem, but this approach does not accurately represent the highly genetically and environmentally diverse human population found in a clinical setting. These studies are further complicated by the many variables that define mitochondrial function.

The ongoing study aims to identify a link between genotype, environment, and mitochondrial function, gaining an insight into their interaction. Here we present the preliminary data for a project investigating mitochondria in the BXD and the AD-BXD isogenic strains. Our experiment is intended to provide detailed, tissue-specific data on the activity of the mitochondrial respiratory chain, reactive oxygen species (ROS) production, and mitochondrial DNA (mtDNA) copy number in mice described by the combination of age, sex, genetic background, and 5XFAD transgene status. We analyze data obtained in our experiment in two ways: on the group level: to identify sex, age or transgene effects on mitochondrial function; and on the strain level: to look for associations with other phenotypes collected in the population, including behavior and neuroanatomy.

Our preliminary data show connection between mtDNA copy number, respiratory activity and BXD strain longevity in 6-month-old animals with the 14-month-old group to be assessed next. We combine the data on mtDNA copy number obtained with a high throughput method of qPCR from many samples and data from a low-throughput method of high-resolution respirometry (HRR). HRR experiments are usually restricted to a small number of experimental groups to process enough samples. In our experiment, in order to be able to compare the HRR against the mtDNA copy number data from all experimental groups we use an incomplete nested design and linear mixed models. Subsequent measurements of ROS production following the same design as in HRR will be added to the respirometry data. This approach allows us to obtain important bioenergetic data and draw conclusions for each grouping factor, addressing a significant limitation within the field of bioenergetics, while capitalizing on the well-defined genetics of the BXD and AD-BXD strains.

In summary, our findings elucidate the mechanisms behind the interactions of genetic makeup, mtDNA copy number, and mitochondrial function in aging and AD, thus paving the road to the development of translatable interventions.

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1486T **Elucidating candidate genetic mechanisms and underlying modifier genes leading to background-dependent tumor growth in the absence of ERBB3 in colorectal cancer** Kaitlyn E Carter, Michael P McGill, David W Threadgill Cell Biology and Genetics, Texas A&M University

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the United States. Significant progress has been made in early detection, diagnosis, and treatment through the developments in precision medicine and increased understanding of the molecular mechanisms governing CRC progression. Overall mortality rates have trended downward over the last five decades but have increased for those under fifty-nine. This study focuses on the need to improve targeted therapeutic approaches based on the genetic alterations of the host and tumor molecular signatures. The ERBB receptor tyrosine kinase family activates genetic mutations and aberrant signaling in pathways. These receptors govern various complex biological functions associated with dysregulated cell proliferation and survival. ERBB3, a pseudo-kinase, lacks intrinsic kinase activity and relies on transactivation of other ERBB receptors. Due to their prominent role in these pathways, the ERBB family

has been investigated as therapeutic targets and seemed promising in preclinical studies. It was shown that intestinal-specific deletion of ERBB3 leads to a significant reduction in colon and intestinal tumor multiplicities. However, the ERBB family performed with little to no efficacy and even promoted tumor growth in some cases during clinical trials. Our group performed additional experiments suggesting that intestinal-specific ERBB3 ablation was genetic background dependent, and that this tumor growth response could be mediated by EGFR (Mantilla-Rojas et al, 2021). This study aims to use novel mouse models to understand the background-dependent tumor growth in the absence of ERBB3 in CRC. By leveraging an F2 population and polyp count distribution, we capture the total phenotypic variance of the 129S1/SvlmJ (129) and C57BL/6J (B6) mouse populations through quantitative trait loci. In doing so, we can better identify molecular signatures or modifier genes and corresponding biomarkers contributing to this effect. We further characterize ERBB3 and EGFR transcript, protein, and protein localization differences in backgrounds using mouse intestinal organoids. ERBB3 and EGFR inhibitor resistance is investigated using mouse intestinal organoids and patient-derived organoids (PDO). We will use these findings to elucidate pathways contributing to tumor progression in the absence of CRC. These studies will broaden our mouse study and better address translational gaps seen in cancer research.

1487T **Machine learning for quantification of behavior in rodent models of aging and Alzheimer's disease.** Joy Afolabi[1], John T Killmar[1], Michael P McDonald[2], Allan G Johnson[3], Robert W Williams[1], David G Ashbrook[1] [1]Genetics, Genomics, and Informatics, University of Tennessee Health Science Center, [2]University of Tennessee Health Science Center, [3]Duke University

Background:

Aging is a major risk factor for many diseases, including Alzheimer's disease (AD). The prevalence of AD continues to be positively correlated with longevity. Alzheimer's disease (AD) is a complex disease, that destroys neurons and brain cells involved in memory and eventually affects reasoning and social behavior. Accurate quantification of behavior is vital for exploring the genetics of these different aspects of AD. Machine learning tools are recently being used to quantify these behaviors.

Research aim:

Mouse models have traditionally been used for behavior assays to test phenotypes including memory, gait and frailty. However, these models have traditionally involved the manual analysis of a limited number of pre-defined behaviors. We aim to use 'computerized' behavior quantification to explain genetic predisposition to aspects of AD and to identify new phenotypes that may be associated with AD and aging in mouse models.

Methods:

We will identify novel behavioral phenotypes associated with aging and Alzheimer's disease within traditional assays, using AD-BXD model mice. We will then use QTL mapping to determine regions of the genome associated with variation in any phenotype measured. Following, we will search for genes within the QTL to identify candidate genes that may influence our trait of interest. We have previously collected videos of mice exploring a snowflake maze. We will use DeepLabCut and a modified version of High-Resolution Net (HRNet) software (supervised learning) as well as Motion Sequencing (MoSeq) or DeepOf (unsupervised learning) in conjunction with Simple Behavioral Analysis (SimBA) software to test if machine learning tools can accurately quantify these behavioral phenotypes of aging and Alzheimer's disease.

Expected Findings:

Alzheimer's disease and aging both lead to a range of linked phenotypes. We aim to identify phenotypes which may be unique to one or shared by both and identify genetic loci underlying these phenotypes.

1488T **CCR6 is required to generate an effective host immune response during *Mycobacterium tuberculosis* infection in mice** Summer Harris[1], Rachel K. Meade[1], Kaley M. Wilburn[1], Alwyn Ecker[1], Oyindamola Adefisayo[1], Ashley Moseman[2], Clare Smith[1] [1]Molecular Genetics and Microbiology, Duke University, [2]Integrative Immunobiology, Duke University

Tuberculosis (TB) remains among the most prevalent infectious diseases in human history and is caused by infection with *Mycobacterium tuberculosis* (Mtb). Mtb is highly successful at infecting and surviving in humans, resulting from a long history of host-pathogen coevolution. TB disease progression is heterogeneous within and between individuals and is impacted by a variety of genetic and environmental risk factors. During Mtb infection, cytokines and chemokines must be carefully balanced to promote bacterial killing or containment without causing excessive inflammation and damage. CCR6 is a chemokine receptor that is expressed by myeloid dendritic cells (DCs), effector and memory T cells, and B cells. CCL20, the only known ligand for CCR6, functions as a chemoattractant that recruits CCR6-expressing immune cells. It has been proposed that CCR6/CCL20 may play a protective role in TB immunity. Here, we sought to establish whether $CCR6^{-/-}$ mice (B6.129P2-

Ccr6^{tm1Dgen}/J) are able to maintain control of Mtb infection. We aerosol infected CCR6$^{-/-}$ mice with Mtb and profiled them at 2, 3 and 4 weeks post-infection. We quantified survival, weight change, bacterial burden and cytokines from the lung, and bacterial dissemination in the spleen. CCR6$^{-/-}$ mice had higher bacterial burden in both the lung and spleen relative to CCR6$^{+/+}$ controls. Additionally, CCR6$^{-/-}$ mice had an increased risk of early death. Overall, this study establishes the functional importance of CCR6 in generating a protective immune response to Mtb.

1489T **Killer toxin K62 of *S. paradoxus* is a novel aerolysin toxin** Jack Creagh Biological Sciences, University of Idaho

Pore-forming proteins are found across the tree of life in various roles, including toxins that kill cells by creating pores, causing uncontrolled ion leakage. Aerolysin toxins are a family of pore-forming toxins commonly found in bacteria, where they function as virulence factors that cause hemorrhagic diseases in animals. Although diverse aerolysins share similar mechanisms of cytotoxicity, they have little to no amino acid sequence conservation. Therefore, the aerolysin family is defined by a five beta-sheet domain, which positions an amphipathic loop in the membrane responsible for membrane pore formation. Using the protein structure prediction software AlphaFold2, we have identified a putative aerolysin toxin named K62, produced by the yeast *Saccharomyces paradoxus*. Our model of K62 has high structural similarity to the bacterial aerolysin-family toxin parasporin-2 with an LDDT over 90 and a root mean squared deviation (RMSD) of 5.5 Å. K62 was first identified as an antifungal "killer" toxin, thought to play a role in niche competition between wild yeasts. K62 is similar to many canonical killer toxins in that it is most active at inhibiting the growth of other fungi at an acidic pH and temperatures less than 30°C. To test our modeling predictions that define K62 as an aerolysin-family toxin, we have developed ectopic expression systems for K62 that recapitulate the antifungal activities of K62. Recombinant K62 forms SDS and heat-resistant complexes with apparent molecular weights of 55 kDa and >250 kDa. These oligomers are considerably larger than the predicted K62 monomer (27.5 kDa) and likely represent stable dimers and possible oligomeric pores. Oligomerization is a crucial step in aerolysin pore formation that is also heat and SDS-resistant due to the prion-like oligomerization of the five-beta sheet domain. Although K62 is an antifungal killer toxin, we have found K62 like sequences in plant pathogenic fungi such as *Fusarium sp.* and the emerging human pathogen *Candida auris*. Given the known role of aerolysins in bacterial pathogenicity, we speculate that K62-like proteins could also be relevant in fungal disease. Using computational, genetic, and biochemical techniques, we have characterized a novel aerolysin family toxin in *Saccharomyces* yeasts that will serve as a powerful model for the future discovery and characterization of diverse aerolysin-family pore-forming toxins.

1490T **Characterization of novel disease-linked mutations in the RNA exosome in *Saccharomyces cerevisiae*** Milo B. Fasken[1], Sara W. Leung[2], Anita H. Corbett[2] [1]Biology, Emory University, [2]Emory University

The RNA exosome is an essential, evolutionarily conserved ribonuclease complex that processes and/or degrades multiple classes of RNA. The 10-subunit complex is composed of an upper ring of three structural 'cap' subunits (Human EXOSC1-3; *S. cerevisiae* Csl4/Rrp4/Rrp40), a lower ring of six structural 'core' subunits (EXOSC4-9; Rrp41/Rrp46/Mtr3/Rrp42/Rrp43/Rrp45), and a catalytic ribonuclease (DIS3/Dis3) at the bottom of the complex. With the assistance of exosome cofactors, RNA substrates are fed through the central channel of the complex to DIS3/Dis3 for processing/decay. Recently, missense mutations in RNA exosome genes *EXOSC1/3/8/9* have been linked to pontocerebellar hypoplasia type 1 (PCH1) and missense mutations in *EXOSC4/5* have been linked to neurodevelopmental disorders. In addition, missense mutations in *EXOSC2*have been linked to a novel syndrome characterized by short stature, hearing loss, retinitis pigmentosa, and distinctive facies (SHRF). To determine the molecular defects associated with missense variants of the human RNA exosome subunits, we and others have developed budding yeast models that express the disease-linked missense variants of the *S. cerevisiae* RNA exosome subunits. To date, these yeast models have shown that several disease-linked subunit variants of the RNA exosome impair yeast growth, reduce RNA processing, decrease subunit levels, and disturb translation. Here, we characterize a novel set of *EXOSC/rrp* mutations in *S. cerevisiae* and compare these to the previously studied *EXOSC2/3/5-rrp4/40/46* yeast models. These studies shed further light on how missense subunit variants of the RNA exosome can alter the function of the complex, potentially in mechanistically distinct ways, and provide insights into the molecular defects that could occur in the cells of affected individuals.

1491T **Human disease modeling in *Xenopus laevis* and *Xenopus tropicalis* at the *Xenopus* Mutant Resource** Zoë T Reynolds[1], Kelsey Coppenrath[1], Sarah Porter[1], Ben Evans[2], Artur Llobet[3], Helen Willsey[4], Gerald Thomsen[5], Richard Behringer[6], Marko Horb[1] [1]Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, [2]Biology, McMaster University, [3]3Laboratory of Neurobiology, Bellvitge Biomedical Research Institute, [4]Department of Psychiatry, University of California San Francisco, [5]Department of Biochemistry and Cell Biology, Stony Brook University, [6]7Department of Molecular Genetics and Program in Genes and Development, University of Texas Houston

At the *Xenopus* Mutant Resource (XMR), we have closely collaborated with the *Xenopus* community to prioritize the generation of null knockout mutants in both *Xenopus tropicalis* and *Xenopus laevis* genes of interest. Generating these

lines at the XMR allows the community to take advantage of our efficient CRISPR pipeline, in addition to the husbandry and lab resources available to help expedite your research project. To date we have generated over 250 mutant lines spanning many research disciplines. My work consists of managing and establishing over 40 mutant gene lines. Specific genes I have targeted have resulted in striking phenotypes. In F0 *X. tropicalis edn3* mutants present we find loss of pigment. *Pax7* null *X. laevis* froglets feature severe bloating around stage 54 of development. We have also created several mutants in genes involved in sex determination, including *dm-w* and *dmrt1*, both of which display a striking phenotype. We have also created *X. tropicalis* mutants *naglu* and *lamp2* are actively being examined for a phenotype due to their role in lysosomal storage and regulation. In this poster, I will present our methodology and progress in generating these various CRISPR knockouts and how the resources at the XMR can help streamline research on these human disease models.

1492T **Functional characterization of the chr1p36.33 *KLHL17*/*NOC2L* pancreatic cancer risk locus suggests effects on the unfolded protein response pathway** Katherine Hullin[1], Ehssan Abdolalizadeh[1], Jun Zhong[1], Jason Hoskins[1], Daina Eiser[1], Michael Mobaraki[1], Aidan O'Brien[1], Ashley Jermusyk[1], Irene Collins[1], Sudipto Das[2], Thorkell Andresson[2], Pancreatic Cancer Cohort Consortium (PanScan)[1], Pancreatic Cancer Case-Control Consortium (PanC4)[1], Rachael Z. Stolzenber-Solomon[1], Allison P. Klein[3,4,5], Jianxin Shi[1], Brian M. Wolpin[6], Jill P. Smith[7], Samuel O. Antwi[8,9], Katelyn E. Connelly *[1], Laufey T. Amundadottir *[1] [1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, [2]Frederick National Laboratory for Cancer Research, [3]Department of Epidemiology, Johns Hopkins School of Public Health, [4]Department of Oncology, Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins School of Medicine, [5]Department of Pathology, Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins School of Medicine, [6]Department of Medical Oncology, Dana-Farber Cancer Institute, [7]Department of Medicine, Georgetown University, [8]Division of Epidemiology, Department of Quantitative Health Sciences, Mayo Clinic, [9]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Mayo Clinic

Pancreatic ductal adenocarcinoma (PDAC) is predicted to become the second leading cause of cancer-related deaths in the US by 2030. Genome wide association studies (GWAS) for PDAC identified chr1p36.33, tagged by rs13303010 (P=7.3x10^{-7}), as a risk locus. Two nearby candidate target genes were implicated by expression quantitative trait locus (eQTL) analysis: *KLHL17* and *NOC2L*. Initial *in vitro* assays to identify variants with allele-specific activity demonstrated enhanced binding and transcriptional regulatory activity for the protective allele. This aligns with an eQTL for *KLHL17* in the Genotype-Tissue Expression (GTEx) v8 dataset, where higher *KLHL17* expression is linearly associated with protective allele dosage (P=4.9x10^{-9}, β = 0.35).

In silico knockdown of *KLHL17* in the pancreas GTEx data reveals lower *KLHL17* is associated with lower expression of heat shock protein genes. We hypothesize that decreased expression of *KLHL17* leads to a reduction in the level of heat shock proteins available to facilitate newly synthesized protein folding leading to increased un/misfolded proteins in the endoplasmic reticulum (ER), ER stress, and dysregulation of the unfolded protein response (UPR). This could result in increased apoptosis, inflammation, and pancreatic cell regeneration, which may promote tumorigenesis.

Under normal conditions, overexpression of *KLHL17* does not confer a growth phenotype, but knockdown of *KLHL17* results in decreased proliferation and increased apoptosis. Under ER stress conditions, this apoptosis phenotype is exacerbated, revealing an interaction effect between *KLHL17* knockdown and ER stress (P=1.16x10^{-6}). We then assessed if the reduction of *KLHL17* expression alters cellular response to ER stress in the normal-derived pancreatic ductal epithelial cell line HPDE. Under ER stress conditions, there is an approximate two-fold increase in spliced *XBP1, HSP5A* and *PERK* mRNA expression when *KLHL17*expression is reduced, suggesting an increased activation of the UPR with reduced *KLHL17* expression.

To determine what role *KLHL17* might be playing in the UPR, we performed KLHL17-FLAG immunoprecipitation followed by mass-spectrometry. This revealed Cullin-3, an E3 ubiquitin ligase, is enriched upon *KLHL17* overexpression and pull-down. Work is ongoing to further elucidate KLHL17's role in pancreatic cells. Understanding how KLHL17 interacts with underlying processes and promotes tumorigenesis may provide insight into potential preventative or therapeutic approaches for patients with a predisposition towards UPR-driven pancreatitis.

1493T **A Yeast Phenomic Model for Mitochondrial DNA Release** Tapasya Katta[1], Ryan Mancinone[2], Keshav K Singh[2], John L Hartman[2] [1]University of Alabama at Birmingham, [2]Department of Genetics, University of Alabama at Birmingham

Release of mitochondrial DNA from the mitochondrial compartment is implicated in the initiation of innate inflammatory responses, mutagenic integration into the nuclear genome, cancer, and related pathologic processes. The first genes to be involved in regulating escape of mitochondrial DNA were identified in *S. cerevisiae*. In particular, *YME1* is an evolutionarily conserved gene that has also been strongly implicated in cancer and is also studied with increasing interest for its role in triggering innate immunity. Other factors in maintaining the mitochondrial outer membrane barrier and in regulating mitochondrial DNA transport across it are highly conserved. We are attempting to more fully characterize evolutionarily

conserved aspects of this gene network, leveraging the mitochondrial escape reporter, together with the *S. cerevisiae* gene deletion strain collection, synthetic genetic array methodology and custom high throughput cell array phenotyping technology developed in our laboratory. The yeast mitochondrial escape (YME) reporter has a CEN element and the TRP1 gene integrated into mitochondrial DNA, so that presence of this modified mitochondrial genome in the nucleus can be detected in a *trp1* auxotroph plated on tryptophan dropout media. We confirmed that the mitochondrial DNA escape phenotype of the *yme1-1* allele segregates as a single gene trait. Other preliminary work on the model has revealed that a point mutation in *YME1* has much greater influence on mitochondrial DNA release than the knockout and ways to enhance the sensitivity of the screen include adding low dose HU and early entry into stationary phase. Our SGA strategy is to first deplete mitochondrial DNA in the yeast gene deletion strain (YGDS) library, and then to introduce the *TRP1*-containing mitochondrial DNA reporter and *trp1* auxotrophy, recovering the haploid YGDS library competent for screening by quantitative high throughput cell array phenotyping. The screen optimization is nearly complete, and we intend to report the results along with consequent hypothesis generation and testing of related hypotheses and other progress during the meeting.

1494T **Genome-wide association study of extended replicative lifespan in outbred populations of *Saccharomyces cerevisiae*** Katie M McHugh, Felipe Barreto, Molly Burke Oregon State University

Longevity varies substantially within and among species, as do heritability estimates for this trait. While molecular genetic techniques have identified many conserved genes and gene regulatory pathways involved in aging and longevity, it is unclear which of these are the most important in regulating observed variation in lifespan within populations. Genome-wide association studies (GWAS) are powerful tools for parsing the relationships between phenotypic and genomic variation, and have been used to investigate the genetic basis of longevity across a number of species, including humans. However, it can be challenging to achieve sufficiently large, unbiased cohorts of long-lived individuals for this approach to be successful in natural populations. Thus, applying GWAS methods to controlled laboratory populations of model organisms can strengthen and supplement the conclusions made in other systems. In this study, we use outcrossed populations of the yeast *Saccharomyces cerevisiae* that harbor extensive standing genetic variation as a GWAS model. Using pooled-population methods, we sequence the genomes and transcriptomes of age-sorted cohorts to investigate the genetic basis of replicative lifespan - the number of times a yeast cell is capable of dividing before death. We show that fluorescence-activated cell sorting (FACS) produces cohorts of cells with significantly differentiated replicative ages, and we use this technique to isolate young and old age cohorts in 12 replicate populations. In each replicate, we compare the genome-wide allele frequencies and global gene expression patterns between the descendants of the young and old cells. We identify candidate alleles that are overrepresented in populations capable of achieving increased replicative ages. We also make connections between observed differences in allele frequencies and observed differences in gene expression to investigate the potential functional consequences of these candidate alleles for replicative lifespan.

1495T **Mutational mapping using dominant synthetic lethal screening to uncover cohesin structure-function relationships** Elizabeth Stephens[1], Nigel O'Neil[2], Peter Stirling[3], Philip Hieter[1] [1]Medical Genetics, Michael Smith Laboratories, University of British Columbia, [2]Michael Smith Laboratories, University of British Columbia, [3]Medical Genetics, BC Cancer Research Center, University of British Columbia

Background: Cancer remains a leading cause of mortality worldwide. Understanding the underlying genetics of a tumour can allow for a personalized approach to exploit genetic vulnerabilities, leading to outcomes with increased effectiveness. One strategy of genetically targeting tumour cells is through synthetic lethal (SL) interactions. A SL interaction between two genes occurs when the disruption of either gene alone is viable, but the disruption of both genes simultaneously results in cell death. Although targeting SL interactions holds immense therapeutic potential, only PARP inhibitors have translated to the clinic. PARP inhibitors are unique in that they cause a gain-of-function (GoF) change in the PARP protein resulting in selective cytotoxicity in BRCA1/2-mutated tumours. The concept of GoF opens new target opportunities for therapeutic design. The cohesin complex (SMC1, SMC3, RAD21, and STAG2) is a compelling target for GoF therapeutic design. Cohesin plays roles in sister chromatid cohesion, DNA damage repair, and chromatin organization and is frequently mutated in a wide range of tumour types. The Hieter Lab demonstrated that cohesin is a conserved SL network hub that interacts with many cancer-associated mutations, including cancer-associated cohesin mutations. One way to cause SL in cohesin-mutated tumours is to further disrupt the cohesin complex.

Approach: *S. cerevisiae* will be used to employ a novel screening approach focused on dominant GoF synthetic lethal genetics. Screening for dominant mutations allows for the study of essential gene targets such as the cohesin complex. Missense mutational mapping will identify states of the target protein, Smc1, that yield more significant effects than loss or partial loss of function to provide insight into druggable pockets for therapeutic design.

Results: Smc1 mutations were screened in a cohesin-compromised strain to identify GoF mutations. This screening revealed 10

key residues all mapping to the ATPase domain, with specific clustering in the signature motif. These mutations are dominant SL with other cohesin subunits and SL in backgrounds with disrupted cohesin levels and replication defects. When mapped to the 3D structure, many of these mutations cluster in ATP interacting regions, revealing a potentially druggable site. The identified mutations will be further characterized to understand the mechanism of dominance, including evaluating their effects on chromosome cohesion, DNA replication and repair.

Significance: Understanding these mutations lays the groundwork for guiding the development of small molecule inhibitors that phenocopy the dominant protein state. My work aims to better translate genetic interactions into anti-cancer SL therapeutics.

1496T **Quantifying tolerance and resistance to fluconazole in *Cryptococcus neoformans*** Jessica Keeran, Lukasz Kozubowski Genetics and Biochemistry, Clemson University

Rates of antifungal drug tolerance and resistance are a growing concern across the globe. Azole antifungal drugs remain an attractive choice in antifungal therapy due to their low toxicity and affordability, an important factor especially in regions where systemic fungal infections are particularly common. Unfortunately, tolerance and resistance to azoles is common due to their mostly inhibitory effect on fungal cells and relatively lower cidal effect. Studies investigating the response of fungal pathogens to azoles are mostly executed in vitro under conditions that are significantly different than the host environment. Therefore, systematic studies of how environment impacts tolerance and resistance to antifungals are essential to improve therapies. The effects of various conditions on tolerance and resistance to the azole drug, fluconazole, have been modeled quantitatively using *Candida albicans*, a common fungal pathogen. However, minimal research has been done to quantify and model azole antifungal drug tolerance and resistance in *Cryptococcus neoformans*. *C. neoformans* is a basidiomycetous pathogenic yeast spread ubiquitously across the globe and is the major causative agent of fungal meningoencephalitis. This pathogen is responsible for approximately 15% of all AIDS-related deaths. One of the most applied antifungal medications for maintenance therapy in cryptococcosis is fluconazole. This study utilizes quantitative techniques that have been previously utilized in *C. albicans* to characterize how various environmental factors impact fluconazole resistance and tolerance in a variety of wild type *C. neoformans* strains. Disk diffusion assays and the image analysis software, diskImageR, were utilized to assess the influence of temperature, media type, and the pretreatment of the cells with various environmental stressors on the development and dynamics of tolerance and resistance to fluconazole. Both temperature and media type significantly impacted *C. neoformans* evolution of resistance and tolerance to fluconazole. Interestingly, some of these effects were also strain specific. Overall, this research displays the complex nature of antifungal drug responses in *C. neoformans*, points the way towards future studies, and could provide insights to help optimize anticryptococcal therapy.

1497T **Overexpression screen of chromosome 21 orthologs in larval zebrafish** Anna J Moyer[1], Claire Conklin[2], Mary Shay Capps[1], Eshika Kudaravalli[2], Summer B Thyme[1] [1]UMass Chan Medical School, [2]University of Alabama at Birmingham

Although Down syndrome is a leading cause of intellectual disability, understanding which chromosome 21 genes contribute to its neurodevelopmental phenotypes remains a critical barrier to developing effective therapies. Larval zebrafish models provide access to high-throughput in vivo brain imaging, behavioral assays, and drug screens that are not possible in Down syndrome mouse models. We leveraged the unique advantages of zebrafish to generate 76 transgenic lines representing 26 human chromosome 21 genes. We are evaluating the neurodevelopmental effects of overexpressing these transgenes using the GAL4/UAS system. A leading candidate gene is *HMGN1*, which competes for binding with histone H1 and promotes chromatin decompaction by binding to the nucleosome core particle. Overexpression of *HMGN1* inhibits Sonic hedgehog signaling, which is disrupted in Down syndrome. To understand how *HMGN1* overexpression affects neurodevelopment in vivo, we cloned cDNAs of three zebrafish HMGN1 homologs (*hmgn6*, *si:ch73-1a9.3*, and *si:ch73-281n10.2*) as well as cDNAs encoding *HMGN1* and *hmgn6* with mutations in the 'RRSARLSA' nucleosome-binding domain. Initial characterization of these lines found that overexpression of hmgn6 using a pan-neuronal driver results in pronounced changes in brain structure and activity, including reduced volume of the midbrain, increased activity of the optic tectum, and reduced activity of the hindbrain. *Hmgn6* overexpression also affected larval behavior, with transgenic fish showing significant disruptions in daytime activity, center preference, and response to acoustic stimuli. Using bulk RNA sequencing, we found that *hmgn6* overexpression affects the expression of transcripts encoding both epigenetic and synaptic proteins. We are currently using single-nucleus RNA sequencing to explore whether overexpression of *HMGN1* homologs disrupts cellular composition or state. Our ultimate aims are to understand how overexpression of trisomic genes affects brain development and behavior in people with trisomy 21 and to identify potential drug targets for treating Down syndrome-associated intellectual disability.

1498T **Progressive motor and non-motor symptoms in park7 knockout zebrafish** Lakshmi Narasimha Murthy Chavali[1], Ingeborg Iddal[2], Ersilia Bifulco[2], Simen Mannsåker[1,1], Dagne Røise[1], Jack O Law[2], Ann-Kristin Frøyset[1], Sushma N Grellscheid[2], Kari E Fladmark[2] [1]Molecular Biology, University of Bergen, [2]University of Bergen

In our exploration of the connection between DJ-1 mutations and early-onset Parkinson's Disease (PD), a disorder marked by a range of motor and non-motor symptoms, we've honed in on DJ-1's pivotal role in shielding against oxidative stress. Building on insights gained from our DJ-1 null fish model displaying diminished tyrosine hydroxylase levels, respiratory mitochondrial failure, and reduced body mass during aging, we delved into the age-related escalation of PD symptoms. Our investigation, encompassing behavioral analyses and scrutiny of mitochondria and associated proteins, uncovered intriguing nuances. While current research underscores a decline in NAD+ as a potential PD contributor, with NAD+ precursor supplementation suggested for slowing disease progression, our study revealed a distinct narrative. In the aging zebrafish, the NAD+/NADH ratio in the brain dipped, but intriguingly, it did not align with behavioral changes induced by DJ-1 loss. Notably, reductions in NAD+ and NADPH manifested later in adulthood. Our findings advocate strongly for the utility of our DJ-1 null fish model, not only in unraveling PD progression intricacies but also as a robust platform for evaluating proposed therapeutic interventions.

1499F **A *C. elegans* Model for Hyperhomocysteinemia: Implications for Disease and Aging** Karli P Sunnergren, Jessica Tanis Biological Sciences, University of Delaware

The amino acid homocysteine is broken down into cystathionine by cystathionine beta-synthase (CBS) or converted to methionine by methionine synthase, which requires vitamin B_{12} as an essential cofactor. Low vitamin B_{12} and CBS deficiency can cause hyperhomocysteinemia, which is characterized by an abnormally high level of total homocysteine in the blood. Hyperhomocysteinemia is a risk factor for cardiovascular disease, vascular dementia, and Alzheimer's disease (AD), although the mechanism underlying the connection between increased homocysteine and these pathologies is not well understood. Loss of cystathionine beta-synthase in mice, flies, and zebrafish causes lethality, preventing the use of these models to understand how elevated homocysteine affects aging and organismal homeostasis. We have knocked out *cbs-1* in C. elegans, which is 54% identical to human *CBS*, creating the first viable model of hyperhomocysteinemia. Loss of *cbs-1* results in increased homocysteine, which is reduced with B_{12} supplementation due to increased conversion of homocysteine to methionine. *cbs-1* mutants exhibit delayed development, reduced brood size, decreased body length, and extended lifespan, all of which return to wild-type levels when the animals are supplemented with B_{12}. Surprisingly, loss of *cbs-1* paralog *cbs-2* does not result in any changes to the aforementioned phenotypes, which may be due to differences in spatiotemporal expression. We have also discovered that *cbs-1* mutants are resistant to paralysis when exposed to the acetylcholinesterase inhibitor aldicarb, suggesting decreased acetylcholine (ACh) in the synaptic cleft. Additionally, *cbs-1* mutants are hypersensitive to the acetylcholine receptor agonist levamisole, indicating compensatory mechanisms on the post-synaptic side of the neuromuscular junction. Together, these results suggest that high levels of homocysteine, resulting from loss of cystathionine beta-synthase, may decrease ACh release from cholinergic neurons, providing new insight into a potential mechanism by which hyperhomocysteinemia impacts risk for AD.

1500F **Nanodrop method for quantification of Orsay virus particle concentration** Jay Ni, Jessica N Sowa West Chester University of Pennsylvania

The natural infection of *C. elegans* by Orsay Virus supports the study of host-pathogen interactions in *Caenorhabditis* species. Knowledge of viral concentration in experiments involving viral infection is necessary for standardization of further downstream processes. Typical assays for virus particle quantification utilize quantitative polymerase chain reaction (qPCR) to amplify viral DNA/RNA for quantification by fluorescence or ELISA for protein detection of the virus. We pursued a different approach to purify and quantify viral particles using the positive-sense RNA virus, Orsay Virus. Our approach features the Thermo Scientific NanoDrop One^{C} Microvolume Spectrophotometer as an alternative to virus quantification by qRT-PCR and other assays.

We have developed a virus prep and purification protocol using an ammonium sulfate precipitation method and Capto Core 700 chromatography beads. The NanoDrop spectrophotometer is utilized to record measurements of protein quantitation with Modified Lowry Assays. Our data is used to determine the viral particle constant and extinction coefficient of Orsay Virus as those values are directly related to protein concentration. A qRT-PCR standard curve of Orsay RNA1 is used for virus quantification. The data from the NanoDrop spectrophotometer will be compared to qRT-PCR assay of viral particle samples. We predict that our UV absorbance method of virus quantification will be an attractive alternative to qRT-PCR and will present the results of the comparison of our spectrometry method versus qRT-PCR.

1501F **The Role of *C. elegans* Metaxins in Mitochondrial Homeostasis** Jonathan Dietz, Eunchan Park, Nathaly Salazar-Vasquez, Nanci Kane, Carol Nowlen, Christopher Rongo Rutgers University

Mitochondria are critical for neuronal function and health, as they are the primary supplier of energy and calcium storage for neurons. Mitochondrial dynamics – fusion, fission, and motility – facilitate energy production and calcium buffering by mitochondria at specific subcellular sites within neurons. Disturbances in mitochondrial function or dynamics contribute to

various neurodegenerative disorders. Using a forward genetic screen in *C. elegans* searching for novel mutants defective in neuronal mitochondrial dynamics, we found that mutations in metaxin 1 (MTX-1), metaxin 2 (MTX-2), and VDAC-1 resulted in fewer mitochondria in *C. elegans* interneuron dendrites. Mammalian metaxin homologs interact with SAM50 to form the sorting and assembly machinery (SAM) complex, which mediates β-barrel protein assembly in the mitochondrial outer membrane (MOM). VDAC-1 is a highly conserved SAM complex substrate that acts as a channel for metabolites across the MOM. We hypothesize that the metaxins promote mitochondrial motility along *C. elegans* interneuron dendrites by mediating assembly of VDAC-1 in the MOM. Mutants for *mtx-1*, *mtx-2*, and *vdac-1* are viable but have reduced lifespans. We found that the mitochondrial unfolded protein response (UPRmt) was activated in *mtx-2* and *vdac-1* mutants, resulting in heat stress resistance and mitohormesis. We are currently investigating the role of *C. elegans* MTX-1 and MTX-2 in MOM β-barrel protein (VDAC-1) assembly and how that impacts neuron integrity.

1502F **The myosin chaperone UNC-45 has an important role in maintaining the structure and function of muscle sarcomeres during adult aging** Courtney J. Matheny[1], Hiroshi Qadota[1], Aaron O. Bailey[2], Andres F. Oberhauser[2], Guy M. Benian[1] [1]Emory University, [2]University of Texas Medical Branch (UTMB)

Sarcopenia, the decline in muscle mass and function without underlying disease, is a major contributor to physical disability and death in the elderly. The molecular mechanisms responsible remain uncertain. *C. elegans* is an excellent model to study muscle sarcomere assembly, maintenance and regulation, and the genetic basis of aging. *C. elegans* undergo an age-dependent decline in animal locomotion and deterioration of muscle sarcomeres, and thus *C. elegans* is a good model for sarcopenia (Herndon et al. 2002). The myosin head domain requires the conserved chaperone UNC-45 for folding after translation and is likely used to re-fold to functionality after thermal or chemical stress induced unfolding. UNC-45 consists of an N-terminal TPR region that binds to Hsp90, and a central region and UCS domain that bind to myosin heads. We observe early onset sarcopenia when UNC-45 is perturbed at the beginning of adulthood, in a ts *unc-45* mutant, indicating that UNC-45 is important during adulthood. During adult aging, there is sequential decline of HSP-90 (day 3), UNC-45 (day 4), and MHC B myosin (day 8). The mRNAs for these proteins decline earlier, on days 1 or 2. Loss of function of one component of the insulin-like signaling pathway, AGE-1 (PI3 kinase), that results in increased longevity has delayed onset of sarcopenia, including maintenance of A-band numbers even at day 16 of adulthood, and delayed loss of HSP-90, UNC-45 and myosin. There is a similar age-related decline of Hsp90 and UNC-45B in mouse skeletal muscle. During the age-dependent loss of UNC-45, there is phosphorylation of UNC-45, beginning at day 3. In day 4, but not day 0, mass spectrometry reveals phosphorylation of 6 serines and 2 threonines, 7 of which occur in the UCS domain, and 2 of which are in residues conserved between nematode and human UNC-45. Of these 7 sites, 3 are on the surface and 4 are buried inside the UCS domain. Molecular dynamics simulations on the known UCS structure indicate that the UCS domain undergoes large scale movements. We predict that the surface residues are likely phosphorylated first, and that buried sites are exposed by structural changes induced by the phosphorylation of the surface residues. Heat shock promoter driven expression of UNC-45 results in sustained levels of UNC-45 and increased MHC B myosin in older animals. Our results suggest that increased expression or activity of UNC-45 might be a strategy for prevention or treatment of sarcopenia.

1503F **Serotonin deficiency from constitutive SKN-1 activation drives a pathogen apathy state** Tripti P. Nair, Brandy P. Weathers, Sean P. Curran University of Southern California

When an organism encounters a pathogen, the host innate immune system is activated to defend against pathogen colonization and the toxic xenobiotics produced. *C. elegans* employ multiple defense systems to ensure survival when exposed to *Pseudomonas aeruginosa* (PA14) including activation of the cytoprotective transcription factor SKN-1/NRF2. Although wildtype *C. elegans* quickly learn to avoid pathogens, here we describe a peculiar apathy to PA14 in animals with constitutive activation of SKN-1 whereby animals choose not to leave while continuing to feed on the pathogen even when a non-pathogenic food option is available. Although lacking the urgency to escape the infectious environment, animals with constitutive SKN-1 activity are not oblivious to the presence of the pathogen and display a typical intestinal distension from PA14 colonization and eventual demise, but surprisingly, fail to learn to avoid pathogen with training. SKN-1 activation, specifically in neurons and intestinal tissues, orchestrates a unique transcriptional program which leads to defects in serotonin signaling from both neurons and non-neuronal tissues that drives the pathogen apathy behavior and pleiotropic responses to selective serotonin reuptake inhibitors (SSRIs). Taken together our work reveals new insights into how animals perceive environmental pathogens and subsequently alter behavior and cellular programs to promote survival.

1504F **Mitochondrial stress in GABAergic neurons non-cell-autonomously regulates organismal health and lifespan in *Caenorhabdities elegans*** Laxmi Rathor, Shayla Curry, Taylor McElroy, Sung Min Han Physiology and Aging, University of Florida

Mitochondrial dysfunction plays a pivotal role in diseases and aging. Recent studies demonstrate that mitochondrial stress in

the nervous system triggers non-cell-autonomous responses in peripheral tissues. However, the specific neurons responding to mitochondrial damage and their impact on health and aging remain unclear. Using *C. elegans*, we investigated the non-cell autonomous effects of mitochondrial stress in gamma-aminobutyric acid (GABA) neurons. Our findings reveal that mitochondrial stress in GABAergic neurons significantly alters organismal lifespan, healthspan, and stress tolerance. These effects coincide with changes in mitochondrial mass, energy production, and reactive oxygen species levels in peripheral tissues. Enhanced DAF-16/FoxO activity is observed in response to mitochondrial stress in GABAergic neurons. This heightened activity is crucial for the observed non-cell autonomous effects on lifespan and stress resistance. Genetic epistasis analysis suggests that GABA neurotransmitter signaling and mitochondrial stress function through the DAF-16/FoxO pathway in a convergent manner, proposing a common mechanism for lifespan extension and healthspan enhancement. Collectively, our data suggested that local mitochondrial dysfunction in GABAergic neurons could influence mitochondria homeostasis, health, and aging of the whole organism through the DAF-16/FoxO pathway.

1505F **Loss of nonsense-mediated mRNA decay and its impact on the *Caenorhabditis elegans* neuromuscular system** Taylor McElroy, Han Gil Kim, Ezgi S. Karabulut, Sung Min Han Physiology and Aging, University of Florida

Aging alters the activity of nonsense-mediated mRNA decay (NMD), a conserved RNA quality control mechanism. However, whether NMD, which degrades both aberrant and some endogenous mRNA transcripts, has a role in age-related motility decline remains undetermined. In *C. elegans*, aging decreases NMD activity in various tissues, including muscle. Several long-lived strains, including *daf-2* mutants, which exhibit enhanced motility later in life, require intact NMD activity for longevity. Thus, we sought to characterize the role of NMD in normal aging and its effects on motility. We show that C. elegans strains with null mutations in components of NMD, including *smg-2*, *smg-5*, and *smg-6*, show decreased movement early in adulthood by reduced speed and body bending in liquid. Markedly, *smg-2* exhibited resistance to aldicarb and levamisole paralysis indicating altered neurotransmission at the neuromuscular junction. *Smg-2* mutants also showed increased neuronal branching in GABA motor neurons at Day 1 of adulthood, suggesting accelerated neuronal aging. Notably, NMD activity declined in GABA neurons of aging wild-type worms. We propose loss of NMD affects age-related motility decline by undermining neuromuscular system integrity. Therapeutics targeting this pathway could improve motility in age-related conditions.

1506F **Adenylosuccinate Lyase Plays a Role in Neuromuscular Coordination** Mia M Peifer, Latisha P Franklin, Wendy Hanna-Rose Biochemistry and Molecular Biology, Pennsylvania State University

Inborn Errors of Purine Metabolism are rare disorders characterized by changes in the activity of enzymes involved in purine synthesis and salvage. The mechanisms underlying the development of their devastating symptoms, including severe neurological and muscular defects, are poorly understood. Understanding these mechanisms is fundamental to developing proper treatment plans and more effective therapeutics.

Adenylosuccinate Lyase (ADSL) Deficiency is one such disorder caused by mutations in the ADSL gene. We model ADSL Deficiency using *C. elegans* with a deletion in *adsl-1* or fed with *adsl-1* RNAi. Disruption of *adsl-1* function results in irregular muscle structure, uncoordinated and slowed swimming movement, slowed crawling, slowed embryonic and post-embryonic development, embryonic lethality, and reproductive defects. We are interested in using the locomotive phenotypes to understand the neurobehavioral functions of *adsl-1*.

C. elegans locomotion is accomplished by the interplay of two key factors: generation of the force required for muscle contraction and coordinated contraction and relaxation of the body wall muscles. Control animals swim with the majority of body bends falling between 61° and 90°. However, an *adsl-1* deficiency results in the randomization of body bend angles with no preference for a specific angle.

Because *adsl-1* have abnormal muscle structure we investigated whether muscular defects are linked to dysregulation of bending angle during locomotion. Using the sarcomere mutant (*unc-95*), which is incapable of generating the force required for normal muscle contraction, we confirmed that loss of muscle integrity causes decreased swimming speeds and thrashing rates, but found that coordination remains unaffected. As an alternative hypothesis, we reasoned that *adsl-1* deficient animals lack the energy required to produce coordinated locomotion given the crucial role *adsl-1* plays in the purine nucleotide cycle, a vital energy maintenance process. Using the mitochondrial complex I mutant (*nuo-6*), we observed decreased swimming speeds and thrashing rates as expected, but no changes in coordination. We argue that the coordination phenotype seen in *adsl-1* deficient animals reflects the role of *adsl-1* in neural function as opposed to muscle function or energy production.

1507F **Transcriptomic Analysis Reveal Modulators of Longevity in an Insulin Growth Factor-1 (IGF-1) "*daf-2*" and Nuclear Hormone Receptor "*daf-12*" Double Mutant in *Caenorhabditis elegans*** Jerald Tan[1], Jan Gruber[1,2] [1]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, [2]Science Divisions, Yale-NUS College

Genetic synergies that extend lifespan involve genes that when mutated, on their own, produce lifespan effects but when impacted together result in amplified effects. One such gene combination is the *daf-2; daf-12* longevity synergy; where *daf-2* mutation extends lifespan *via daf-16* FOXO transcription activation by 1.5 to 2-fold while *daf-12* mutants do not extend lifespan. When mutated together, the resulting lifespan extension versus wild-type is close to 3-fold. In the attempt to understand what drives this longevity, we performed a transcriptomic analysis on a canonical allele *daf-2 (e1370)* and heterochronic allele *daf-12 (m25)* as a *daf-2 (e1370); daf-12 (m25)* double mutant. We found that out of 23,435 Differentially Expressed Genes (DEGs), only a small subset of genes were specific to this double mutant. We validated these results *via* RNAi knockdown experiments for lifespan survival. Our results revealed that the genes identified by transcriptomics promote lifespan extension beyond these double mutants without affecting reproductive capacity of the lifespan-extended animals. These results suggest that altering these genes may have an impact in driving longevity in this long-lived *daf-2; daf-12* mutant model.

1508F **Reproductive system aging in the canonically "non-aging" *C. elegans* dauer** Fred Koitz, Kacy Lynn Gordon Biology, University of North Carolina at Chapel Hill

Stressful conditions early in development are often correlated with shortened lifespans; however, the *C. elegans* dauer larval stage is induced by early life stress and is a diapause state in which development–and apparently aging–ceases. Entering dauer diapause can extend an organism's total lifespan many times over, with time spent in dauer not affecting the animal's post-recovery lifespan. Dauer is induced by starvation (among other triggers) and pauses development until conditions are more favorable for reproduction. While much research on dauer has focused on its effects on lifespan, it is the preservation of reproductive ability that makes dauer adaptative.

An overlooked finding is that animals that spend longer periods in dauer have a higher incidence of later reproductive defects and reduced fertility. This conflicts with the notion of dauer as "non-aging," as aging involves a time-correlated deterioration of internal physiology. As the reason for this reproductive system decline in extended dauers is unknown, we set out to identify reproductive system changes during dauer that could explain this post-dauer reproductive defect using genetic analysis and live-imaging of dauers to examine changes to the reproductive system over the span of six weeks (a duration equal to 2-3 standard *C. elegans* lifetimes).

We discovered that there is a dramatic reduction in germline cell number as dauer duration increases, correlated to a reduction of overall gonad size. Remarkably, we observed that the number of germ cell nuclei per gonad arm never declined to fewer than two germ cells. The dauer constitutive mutant for an insulin receptor gene, *daf-2(e1370),* did not recapitulate the germ cell reduction seen in starvation-induced dauers. Thus, germ cell reduction requires insulin signaling, and it is not simply a consequence of accumulated damage from prolonged starvation during the non-eating dauer stage.

We hypothesize that germ cell reduction is a regulated response to starvation that protects the surviving germ cells, and we are working to identify its genetic regulators. The decline of reproductive system components over time in dauer argues against the "non-aging" dauer paradigm, and could illuminate how organisms modify their reproductive capacity in the face of stresses such as starvation.

1509F **The Role of MicroRNA-71 in a Model of PolyQ Toxicity** Olivia C Dempson[1], Alexandre de Lencastre[2] [1]Quinnipiac University, [2]Biology, Quinnipiac University

Huntington's Disease is a neurodegenerative disorder associated with polyglutamine (PolyQ) repeats in the Huntingtin gene (HTT). Expression of these repeats leads to misfolding of the protein and aggregation in neurons. MicroRNAs (miRNAs) are non-coding RNAs that span 22 nucleotides and have been associated with the regulation of gene expression. Previous research has shown that miR-71 is a pro-longevity gene in *C. elegans* and delays phenotypes associated with the expression of proteins containing long polyglutamine (polyQ) repeats. In *C. elegans* strains expressing proteins in the muscle containing polyQ repeats fused to a fluorescent protein (Q35 and Q40), miR-71 overexpression rescues the motility defects associated with PolyQ expression. This study analyzes the influence of miR-71 in *C. elegans* expressing longer repeats in neurons, such as nQ67. To observe the influence of miR-71 on polyglutamine expression in neurons, we measured the motility of double mutant strains that express a pan-neuronal fluorescent protein that contains 67 repeats of Q (nQ67) and overexpress miR-71. This study also aimed to determine if the DNA replication inhibitor FUdR may be used during these motility assays. The results suggest that FUdR may be used in these strains and that overexpression of miR-71 can rescue *C. elegans* strains expressing polyQ67 in neurons.

1510F **Community connections power health disparities research** Ebony Dyson[1], Drea Darby[1,2], Dara Ruiz-Whalen[1], Alana M O'Reilly[3] [1]eCLOSE Institute, [2]Department of Entomology, Cornell University, [3]Fox Chase Cancer Center

Cancer, diabetes, mental health, infectious disease and other preventable health conditions are more common and more lethal in communities lacking representation in biomedical research. Despite demonstration of the existence of these health disparities over decades, little progress has been made. We created the eCLOSE Undergraduate Bridge to Research Program to begin to address health disparities prevalent in communities represented by program participants. Senior high school students, undergraduates, and recent college grads with limited research experience first learn laboratory techniques in the context of research projects leveraging Drosophila models of diseases of personal importance. After completion of "Bootcamp", students create independent projects that address the community health need of highest interest, using molecular genetics, developmental biology, educational interventions, and biochemistry. A project highlight focuses on HPV-dependent cancers in underserved communities, with more than a dozen student participants contributing insights to a testable, molecular model for prevention of HPV disease. Generous grants from FlyBoard have supported 14 UG Bridge participants since 2019.

1511F **Investigating Presenilin as a Potential Molecular Target of Opportunity for Exploring Alzheimer's Disease** Christopher Ramirez, Maria Lourdes Mendoza Aragon, Laura Galvan, Judy Especial Abuel, Blake Riggs Biology, San Francisco State University

Alzheimer's disease (AD) is a prevalent neurodegenerative disease affecting 6.7 million Americans aged 65+ in 2023, with projections to triple by 2060. Current treatments primarily slow disease progression, emphasizing the need for novel therapeutic strategies. We investigate Presenilin (PSN) as a promising therapeutic target in AD. While PSN's signaling function is recognized, its role in neurodevelopment, particularly its regulation of Tau expression via Notch/Amyloid Precursor Protein (APP) signaling, remains elusive. We hypothesize that PSN defects can induce AD-like symptoms. We use *Drosophila melanogaster*, with its versatility for genetic studies, to investigate PSN's neurodevelopmental role and its influence on neural behavior. We will examine PSN mutant lines in Drosophila larval brains for any defects that resemble a Tau-like phenotype. Our approach involves immunohistochemistry to identify AD-like markers with PSN lines in 3rd Instar larvae brains. Using a STELLARIS Confocal Microscope and Imaris software, we will differentiate and count neuronal, glial, and mushroom body cells. Expected results may reveal behavioral variances between controls, including differences in negative geotaxis, larval movement, and feeding behaviors. Our findings seek to highlight PSN's vital neurodevelopmental role and its potential as an AD therapeutic target, offering insights into improved treatments for neurodegenerative disorders worldwide.

1512F **Optimizing NADPH assay conditions for analysis of a CRISPR screen for *Zwischenferment* (*Zw*)/Glucose-6-Phosphate Dehydrogenase (G6PD) mutations in *Drosophila melanogaster*** Jason Hare, Alexis Nagengast Biochemistry, Widener University

Glucose-6-phosphate dehydrogenase (G6PD) is the most common enzyme deficiency in humans, affecting 400 million people worldwide. The *Drosophila Zw* (*Zwischenferment*) gene codes for the conserved G6PD enzyme that is the rate limiting step in the pentose phosphate pathway (PPP). The PPP provides NADPH required for lipid biosynthesis under high nutrient conditions and NADPH levels can be measured to assay G6PD enzyme activity. In both humans and *Drosophila,* G6PD is alternatively spliced in the 5' UTR (untranslated region) to produce two different protein products that vary in length and initial N terminal sequence. Isoform A results in the longer protein product and Isoform B/C produces the shorter product yet the functional significance of this is not fully understood. A CRISPR/Cas9 screen targeted the Isoform A coding sequence to allow for the characterization of Isoform B independently and this resulted in several different frameshift mutations. Additionally, sequencing of the parental fly lines *yw* and *FM7* revealed a frameshift deletion mutation in *FM7* predicted to also result in a premature truncation of Isoform A. The G6PD enzyme activity levels in these different fly lines will be assayed to better understand the functional significance of the products of Isoform A and B.

1513F **Kefir Treatment in a Parkinson's Disease Model of Drosophila Melanogaster** Rachael B.D. Triglia, Regina Lamendella, Kathryn A. Jewett Biology, Juniata College

Parkinson's disease (PD) is a neurodegenerative condition characterized by the deterioration of motor skills, sleep, memory changes, and the accumulation of irregular protein clusters. Increasingly, the gut microbiome and dietary choices have been found to influence physical and cognitive health. New research indicates that probiotics may present a pathway for promoting a healthier lifestyle and potentially managing PD symptoms. Several studies have explored the impact of manipulating the gut microbiome in humans with PD with results showing enhanced mobility and improved sleep patterns.

The gut-brain axis, or gut-brain connection represents a bidirectional connection between the enteric nervous system and the central nervous system. The introduction of probiotics into one's diet directly influences this network. To explore how dietary factors might affect PD-like symptoms in a *Drosophila melanogaster* model, adult fruit flies will be provided food treated with kefir, a probiotic made of 12 active cultures and over 20 billion colony forming units. We hypothesize that manipulation of the gut microbiome will reduce the PD-like symptoms of the flies.

Previous research has shown positive outcomes when manipulating the diet of *Drosophila* in an Alzheimer's disease model

(Batista et al., 2021, Sci Rep 11:11262). This study compares healthy flies (RV) and sick flies (GBA del) expressing PD-like symptoms (due to the deletion of PD genetic risk factor gene GBA encoding the enzyme glucocerebrosidase). Adult flies will be placed on untreated food as a control group and their performance compared with flies placed on kefir-treated food. Flies will be assayed to determine whether kefir-infused food enhances their climbing abilities, extends their life span, normalizes sleep, and decreases the accumulated protein aggregation. Additionally, the gut bacteria of each group will be analyzed through gut dissections subjected to 16S rRNA gene sequencing. The kefir will also be sequenced through 16S rRNA gene sequencing. We hypothesize that flies on the kefir-treated food will exhibit improved climbing abilities, extended lifespans, normalized sleep, and protein aggregation (measured by western blot) will be decreased in the kefir-treated groups. If correct, researching kefir as a potential treatment to mitigate the symptoms of Parkinson's Disease in human patients could be the next step for experts studying neurodegenerative diseases.

1514F **Understanding changes to sleep behavior and gene expression caused by glucocerebrosidase deficiency in a *Drosophila melanogaster* model of Parkinson's disease** Jason Brandon[1], Sam Anderson[2], Regina Lamendella[1,2], Kathryn A. Jewett[1] [1]Biology, Juniata College, [2]Contamination Source Identification (CSI)

Sleep/wake cycles within many animals, including *Drosophila melanogaster*, is a complex, and not yet fully understood phenomena. Sleep disorders from associated neurodegenerative diseases, such as Parkinson's disease (PD), stem from alterations in cellular processes, sometimes altered because of a genetic mutation. Through this project, we seek to develop an understanding into how genes with a role in sleep regulation that are also associated with extracellular vesicles (EVs) may play a part in PD.

EVs are small, membrane-bound particles released by cells into the extracellular environment. PD can be characterized by the development of protein aggregation within the brain, which can disrupt endosomal functions such as the release of EVs. A genetic form of PD is associated with mutations in the *GBA* gene which encodes a lysosomal lipid-modifying enzyme, glucocerebrosidase. *GBA* deletion in *Drosophila* leads to lipid alterations, increased protein aggregation, changes in EVs, and sleep alterations. When *GBA* expression is rescued in specific cell types, abnormal protein aggregation decreases.

Using western blot techniques, we will assess protein aggregation in healthy and mutant (homozygous *GBA* deletion) flies as well as the effects of glial cell-specific expression of *GBA* in both groups. Preliminary data suggests that glial cell *GBA* expression is sufficient to rescue protein aggregation. Using the Drosophila Activity Monitoring System (DAMS), sleep differences were observed between healthy and mutant flies, specifically in their response to temperature-induced sleep deprivation. The sleep of healthy and mutant flies with and without glial cell *GBA* expression will be analyzed as well.

Using The Database for Annotation, Visualization, and Integrated Discovery (DAVID), enriched genes between two pathways of interest, sleep homeostasis and extracellular vesicle biogenesis have been identified. We are using these results to target specific genes, with RT-qPCR to quantify expression differences among healthy and mutant flies with and without glial cell *GBA* expression. We predict changes in gene expression to reflect the observed changes in sleep and EVs seen in healthy and mutant flies.

1515F **Identifying and characterizing CRISPR/Cas9 medicated mutations in *Zwischenferment* (*Zw*), the *Drosophila* ortholog of Glucose-6-Phosphate Dehydrogenase (G6PD)** Katharyn Mackiewicz[1], Kathleen Miller[2], Morgan Grace[1], Michael Toneff[1,2], Alexis Nagengast[1] [1]Biochemistry, Widener University, [2]Biology, Widener University

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in humans. It is a non-lethal mutation that causes acute hemolytic anemia which is where the red blood cells are broken down more rapidly than they are produced. The *Zwizchenferment* (*Zw*) ortholog, which is used to study the role of G6PD in *Drosophila melanogaster*, has an alternative splicing pattern that is conserved between *Drosophila melanogaster* and humans. This splicing pattern results in three isoforms of the gene and leads to two different protein products that vary in length and have an alternative N-terminal protein sequence. The significance of these protein products is not fully understood. CRISPR/Cas9 screens were conducted to knockout isoform A and study the functional significance of isoform B/C. PCR and sequencing of the established stocks identified two stocks with a two-base deletion, one stock with a one-base deletion, and one stock with a one-base insertion. These deletions and insertions caused frameshift mutations that result in an early stop codon and possible prematurely truncated protein products. This project aims to investigate the function of each isoform in minimizing reactive oxygen species (ROS) damage. Future work will test the significance of the alternative protein products on minimizing ROS damage through the use of paraquat challenges. These challenges will look at the difference in survival between fly lines with and without a CRISPR-mediated mutation in hopes to better understand treatment for this deficiency in humans.

1516F **Extracellular Vesicle Protein Aggregation Analysis within a *Drosophila melanogaster* Neural Cell Culture Model of Parkinson›s Disease** Allison M. Johnston, Kathryn A. Jewett Department of Biology, Juniata College

The *GBA* gene encodes for the enzyme, glucocerebrosidase which is associated with lipid modifications and resides within the lysosome. Mutations of *GBA* are commonly associated with Parkinson's disease (PD) conveying an increased disease risk. Since PD is known to be a neurogenerative disease with protein aggregation causing deficits within the brain, and both humans and *Drosophila melanogaster* naturally have the *GBA* gene, studying the effects of *GBA deletion* within *Drosophila* models can give us a unique insight on the neural effects of protein aggregation. When *GBA* is ubiquitously deleted in *Drosophila* the flies have a shortened lifespan and protein aggregation can be found in the brain, muscle tissue and extracellular vesicles. Extracellular vesicles produced and sent by various cell types *in vivo* may explain how protein aggregation spreads throughout the body and brain during neurodegenerative disease states. In this study, we aim to establish primary *Drosophila* neural cell cultures as a viable option for studying GBA-associated PD and to assay the presence and effect of protein aggregation within extracellular vesicles isolated from culture media.

1517F **Immune-stimulation of Drosophila larval hemocytes drives the rapid generation of lipid droplets via an NFkB and DGAT1 dependent pathway** Roger White[1], Asmita Dutta[1], Jinghong Tang[1], Neal Silverman[2], Michael Welte[1] [1]University of Rochester, [2]University of Massachusetts Medical School

Lipid droplets (LDs) play critical roles in energy homeostasis, lipid metabolism, and protein sequestration in numerous cell types. It is increasingly recognized that they also have important functions in the immune system, but in most cases the underlying mechanisms remain to be characterized. We discovered that exposing Drosophila larvae to *E. coli* by gentle pricking leads to LD accumulation in hemocytes, fly macrophages. Within just three hours, LD numbers double. This rapid, massive accumulation suggests that these LDs might play a role in the immune response. We observe similar LD accumulation when using heat-killed bacteria or the bacterial cell-wall component peptidoglycan (PGN), implicating the IMD pathway in this response. Indeed, in mutants for the NFkB family member Relish LD accumulation is abolished. Further, we find that LD accumulation is cell autonomous in hemocytes. First, hemocyte-specific knockdown of the PGN receptor PGRP-LC severely blunts the response, and hemocytes exposed to PGN *in vitro* accumulate more LDs. The increase in LD number is likely due to new LD biogenesis as PGN stimulation leads to the appearance of tiny LDs and these LDs are highly enriched for the marker LiveDrop, a protein that relocalizes from the ER to newly emerging LDs. From these data, we conclude that PGN drives the formation of new LDs. In many cells, triglyceride synthesis is the rate limiting step for LD biogenesis. Indeed, in mutants for the triglyceride synthase DGAT1/Midway, LD production upon PGN challenge is indistinguishable from controls exposed to buffer. We conclude that the immune-induced LDs contain mostly triglyceride and are due to new LD biosynthesis. We are now testing candidate lipid metabolism genes induced upon bacterial infection to determine if their upregulation mediates LD accumulation after PGN treatment. The long-term goal is to specifically prevent immune-induced LDs in hemocytes to be able to address which immune functions might depend on these LDs.

1518F **Deciphering the Molecular and Cellular Mechanisms Governing Tissue-Selectivity of *Ras* Oncogenic Mutations** Takuya Akiyama[1], Matthew C Gibson[2] [1]Biology, Indiana State University, [2]Stowers Institute for Medical Research

Mutations in *Ras* are detected in various cancers. Ras family proteins play pivotal roles as small GTPases, orchestrating cellular growth and cytoskeleton remodeling. Comprehensive cancer genome sequencing unveils that certain *Ras* mutations appear more frequently in specific tissues. Deciphering the molecular and cellular basis underlying the emergence of this tissue selectivity can have significant implications for cancer initiation and progression. To investigate the impact of *Ras* mutations on cancer development, we established inducible alleles by leveraging CRISPR/Cas9 genome editing. In the wing primordial tissue, introducing a mild *Ras* oncogenic mutation did not affect the tissue architecture, whereas a strong mutation disrupted it by forming cysts. In the adult intestine, cells carrying the mild oncogenic mutation had a growth advantage compared to *wild-type* cells, ultimately taking over the intestine and causing intestinal barrier dysfunction. As expected, when we induced the strong *Ras* mutation, the mutant cells hyper-proliferated and rapidly occupied almost the entire intestinal tissue. However, soon after the clonal expansion of mutant cells, they were eliminated from the gut epithelia, restoring *wild-type* cells in the intestine. Our results indicate that the strong *Ras* oncogenic mutation is counterintuitively less harmful than the mild mutation in the intestine due to the allele-dependent clonal regression. These distinct mutant cell behaviors likely contribute to the tissue-selectivity of *Ras* oncogenic mutations. Our findings also suggest that a partial reduction of *Ras* oncogenic activity by any means, such as drug treatment, has a detrimental effect by prolonging the persistence of mutant cells in some contexts.

1519F **Neuronal expression of the P3 peptide (Aβ17-42) in Drosophila has deleterious effects on lifespan, behavior, degeneration, and gene expression and exacerbates the effects of full-length Aβ1-42** Jacquelene Hundelt, Ginger Holmer, Megan Saefong, Rebekah Larreynaga, Hannah Moalla, Emily Broutian, Jeremy Lee University of California: Santa Cruz

Alzheimer's disease (AD) is a neurological disorder that leads to a loss of cognitive function and memory. Approximately 1 in 9 people in the United States 65 years old and older have AD. An important protein in AD is the amyloid precursor protein (APP), which is expressed in neurons. APP is cleaved in two major ways. It can be cleaved by β-secretase and then ɣ-secretase to

produce the 42-amino acid peptide, amyloid beta (Aβ). An alternative pathway involves cleaving APP by α-secretase and then ɣ-secretase to produce a 26-amino acid peptide, called P3 (aka Aβ 17-42). P3 is a truncated form of Aβ and is 16 amino acids shorter than Aβ (Aβ 1-42). Research has shown that Aβ aggregates into oligomers, fibrils, and plaques that disrupt neuron function. Recent research indicates that P3 is similarly amyloidogenic and is cytotoxic in vitro.

Our experiments focus on P3 to determine whether it has similar effects on neurons as Aβ in vivo. In our experiments, we compare the effects of P3 to those of Aβ in Drosophila expressing the human peptides pan-neuronally, using the APPL-Gal4 driver. A longevity assay was conducted to assess the effects of Aβ and P3 on lifespan. Results indicated that P3, when expressed alone, decreases lifespan, and, when expressed with Aβ, exacerbates Aβ's negative effects. A Rapid Iterative Negative Geotaxis (RING) assay was also conducted to analyze the effects of the expression of both peptides. We found that P3 and Aβ affect the flies' locomotive ability. Furthermore, scanning electron microscope imaging was carried out on the eyes of flies expressing these peptides under the GMR-Gal4 driver. Our results showed that both P3- and Aβ-expressing flies had eye bristle and ommatidial defects, with the most substantial impacts in flies co-expressing the peptides. All these results indicate that P3 has similar but less severe effects as Aβ on neurons and exacerbates Aβ's effects when co-expressed with P3. RNA sequencing was performed on flies pan-neuronally expressing these peptides. This revealed that expression of Aβ and P3 had similar down-regulatory effects on gene expression, especially of proteolytic genes. We are now conducting olfactory assays to assess the effects of the expression of both peptides on learning and memory. Our next step will be to conduct coimmunoprecipitation experiments to determine whether P3 interacts with Aβ directly, so it might contribute to Aβ aggregation and its pathological effects.

1520F **Role of neuronal tRNA processing enzyme in the formation of mushroom bodies in *Drosophila*** Saathvika Rajamani, Lucia Vilchez, Edward Dubrovsky Biological Sciences, Fordham University

RNase Z is a highly conserved gene that codes for an endonuclease playing an important role in the maturation of tRNA molecules. Mutations in the human homolog of this gene have been associated with variable neurological presentations ranging from seizures and neurodevelopmental delay to microcephaly and ataxia. As a first step in understanding the mechanism underlying the associated neuropathology, we generated fly models with neuron-specific RNase Z knockout using CRISPR/Cas9 technology. This disruption led to severely reduced longevity and loss of motor activity, accompanied by smaller sized brains in both larval and adult stages. One of the most evident observations was the loss of morphologically distinct structures in the adult brain, specifically the absence of neurite tracts surrounding the mushroom bodies (MBs). MBs are a pair of neuropils in the central brain that mediate olfactory learning and memory in insects. Further investigation with an antibody specific to the MB revealed several aberrant phenotypes in the adult knockout brains; these defects included truncated, thin or missing α lobes, and fused or missing β lobes. The damage was less extensive in the brains expressing a disease variant, Thr494Ile, with the defective morphologies limited to thinner α lobes and fused β lobes. We hypothesize that the loss of lobes could be due to improper neurogenesis. To explore this, we studied the MB phenotypes at pre-adult stages. Surprisingly, we saw no difference in the lobe morphology in the knockout or mutant larval brains when compared to the controls; this finding indicates that the abnormal morphologies in the adults stemmed from the need for neuronal RNase Z function, specifically during the metamorphic transition. Given that abnormal MB morphology has been linked to impaired cognitive ability, we also hypothesize that flies harboring mutant forms of neuronal RNase Z will show defective learning and memory retention. Altogether, our research suggests a critical role for RNase Z in proper mushroom body formation with a potential link to intellectual disability.

1521F **Characterizing the effects of obesogenic diets on Drosophila *melanogaster* immune responses across control lines** David J Duncan Biological Sciences, University of South Carolina

Diet impacts organismal health by providing macromolecules to support cellular survival and function. Obesogenic, or high-fat and high-sugar, diets can lead to long-term detrimental health effects including multiple cancer types, type 2 diabetes, insulin resistance and excess body fat. Over 60% of adults in the United States are overweight or obese and 42% of obese individuals also present with chronic inflammation. Despite this association, the cellular and molecular mechanisms that link obesity and associated diseases remain unclear. We use *Drosophila melanogaster* as an *in vivo* model to better understand the effects of obesogenic diets on the immune response and begin examining the potential therapeutic effects of dietary plant compounds on obesity-associated chronic inflammation. Given the broad range of "standard" diets and "control" fly lines used in the field, we are first characterizing lifespan and reproduction of several control lines across commercially and non-commercially available foods to identify an optimal-base diet. Thus far, we find there is a difference in lifespan and reproduction patterns across the different control lines fed different standard diets. Oregon-R and yw control lines have a longer lifespan on a diet in which the sugar source is molasses, while w^{1118} control line has the shortest lifespan. A high fecundity rate was observed in yw control line fed a corn syrup-based diet and molasses diet. While molasses diet supports higher egg production for all control lines. We will also identify an obesogenic dietary condition that induces immune responses in adult flies by measuring immune

response pathway activity and antimicrobial peptide production in flies fed high-sugar or high-fat diets. In future studies, we will use this approach to screen botanical supplements for their ability to suppress the diet-activated immune response. We hope that this work will inform the development of treatments for diet-induced obesity-associated diseases in humans.

1522F **Blue light shortens development and longevity across generations in *Drosophila melanogaster*** Monica Andrea Lopez Bautista, Viviana Matilde Mesa Cornejo, Jorge Enrique Mejía Sánchez Centro Universitario de los Lagos, Universidad de Guadalajara

Ambient light affects multiple biological and behavioral functions in living beings that have set the guideline for their evolution and development; however, the growing use of artificial light, coming from light-emitting diodes (LED), has marked a new paradigm, in which all organisms have been involved. Although LED technology represents a great advance worldwide due to its energy efficiency, useful life and cost, it should be reported that chronic exposure over a long period of time, especially in the blue range of the visible light spectrum, causes desynchronization of the circadian cycle, which can impact sleep imbalance, hormonal disorders, mood, among others. Therefore, in this work it was evaluated the effect of blue light (**λ**: 457.33 ± 45 nm) at an illuminance of 100 Lux across five generations in two strains of the model organism, *Drosophila melanogaster* (*Canton-S* and w^{118}) in four different photoperiods: 12:12 LD (Light: darkness), 16:8 LD, LL (constant lighting) and DD (constant darkness), to observe their behavior at the level of development of each larval and pupal stage, reproduction, courtship and longevity. The results confirm that exposure to blue light exerts a toxic effect on the development of the fly regardless of the type of photopigments that they lack both at the level of reproduction and longevity and is maximized throughout each generation. On the other hand, the w^{118} strain showed a higher population rate compared to the wild-type strain, except for that obtained in the LL treatment. In fact, it has been determined that throughout the various stages of development in the fly, these can be accelerated or slowed down by being chronically exposed to blue light depending on the strain. These results conclude that although the flies are exposed to a low intensity of illuminance, its cumulative effect across five generations exerted a significant change depending on the time of exposure to blue light.

1523F **Hippo-activated cells induce non-cell autonomous carcinogenesis in *Drosophila*** Daichi Honda[1], Misako Okumura[1], Chisako Sakuma[2], Masayuki Miura[3], Takahiro Chihara[1] [1]Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University, [2]RIKEN Center for Biosystems Dynamics Research, [3]Graduate School of Pharmaceutical Sciences, The University of Tokyo

The Hippo pathway is known as the tumor suppressor pathway and overwhelming studies indicate that inhibition of the Hippo pathway leads to excessive cell proliferation and carcinogenesis. However, the genetic analysis of cancer has revealed that the Hippo pathway is activated in some cancers, suggesting that activation of the Hippo pathway may be involved in carcinogenesis and that the Hippo pathway has a paradoxical role in carcinogenesis. Therefore, we decided to investigate the possibility that activation of the Hippo pathway contributes to carcinogenesis.

First, we genetically induced Hippo-activated cells in the *Drosophila* wing disc (epithelial tissue) by knocking down or overexpressing the Hippo-pathway components (*strip*, *warts*, and *yorkie*), and examined the effects on two cancer markers, the activity of the mTOR pathway and MMP1 expression. Hippo-activated cells upregulated these cancer markers in their surrounding cells, thereby transforming the surrounding cells into “tumor-like cells”, suggesting that Hippo-activated cells become a “tumor-inducing center”. Second, we analyzed how Hippo-activated cells cause non-cell-autonomous activation of the mTOR pathway by inhibiting downstream signaling of the Hippo pathway (apoptosis signaling). We found that the upstream regulator (Dronc: initiator Caspase) but not the downstream regulator (Dcp-1, etc.: executor Caspase) of apoptosis signaling is required for non-cell-autonomous activation of the mTOR pathway. We hypothesize that the initiator Caspase would regulate the expression or secretion of secretory molecules that activate the mTOR pathway in their surrounding cells. In addition, we conducted genomic deficiency screening to identify the regulator of the induction of tumor-like /mTOR-activated cells and discovered the uncharacterized amino acid transporters, which we named *sat1/2*. In the *sat1/2* mutant background, Hippo-activated cells could not effectively induce the mTOR-activated cells. We hypothesize that Hippo-activated cells can use amino acids to cause carcinogenesis in their surrounding cells. We will discuss the possible mechanism underlying carcinogenesis caused by activating the Hippo pathway.

1524F **Investigating the role of Pvr signaling in myotonic dystrophy type 1** Delaney Baratka, Madeline Brunt, Ginny R Morriss Biological Sciences, University of Mary Washington

Myotonic Dystrophy Type One, DM1, is a progressive, multisystemic, and autosomal dominant disorder caused by expansion of CTG-repeats in the 3’-UTR of the DMPK gene. While we understand the causal mutation that leads to DM1, the underlying mechanisms of disease progression remain unclear. Prior research in a mouse model of DM1 identified deregulated PDGFR- β signaling. PDGFR-β plays a role in cell survival and differentiation. Another study implicated the ligand for Pvr, the Drosophila ortholog of PDGFR-β, as a modifier of DM1 phenotypes. This study will evaluate the role of the Pvr signaling pathway in the

Drosophila DM1 model. The Drosophila DM1 model uses the Gal4/UAS system to express the expanded CUG repeats. We plan to modulate the expression of pvr by RNAi knock down (pvr-RNAi) and overexpression (Pvr-OE) in flies with and without the CUG-repeat expression (CUGexp). A mating scheme has been set up to obtain flies driving expression of CUGexp RNA, pvr-RNAi, Pvr-OE, CUGexp RNA + pvr-RNAi, and CUGexp + Pvr-OE using the Mef2-Gal4 driver. The use of the fruit fly model allows us to study the signaling pathway in a more simplified view to understand its role in both normal muscle function and in DM1. Our main goal is to determine the role of Pvr signaling for proper skeletal muscle function and in DM1 pathology. A secondary goal is to determine whether overexpression of Pvr in flies affected by DM1 can rescue the DM1 muscle phenotype.

1525F **Assessing progression of muscle phenotypes in an adult-onset model of myotonic dystrophy in Drosophila melanogaster** Kayla Rodriguez, Ashlyn Rauch, Ginny R Morriss Biological Sciences, University of Mary Washington

Myotonic Dystrophy Type One (DM1) is a multisystemic disorder caused by expansion of CTG repeats in the dystrophia myotonica protein kinase (DMPK) gene. Individuals diagnosed with DM1 may display a wide spectrum of CTG expansions, ranging from 50 to 4000 repeats. Unaffected individuals typically have between 5 to 37 CTG repeats. In individuals with shorter repeat expansions, development of symptoms typically occurs in the later adult years, where individuals with very long expansions can have a juvenile or congenital onset. Individuals diagnosed with the congenital form of myotonic dystrophy commonly exhibit developmental defects, in addition to some of the classical DM1 symptoms. Since DM1 results in progressive worsening of symptoms over generations, it is important to understand the progression of the disease. The Drosophila DM1 model uses the GAL4/UAS system to drive expression of expanded CUG repeat-containing RNA (CUGexp). Typically, GAL4 drivers that have been used have shown severe muscle atrophy, reminiscent of human DM1, but these drivers are expressed embryonically, making it difficult to tease apart defects that are due to repeat expression and defects that are due to impaired development. This study uses the 1151-Gal4 driver to study the progression of skeletal muscle weakening and wasting. The onset of expression of the 1151-Gal4 driver is during the pupal stage, we are able to understand the progression of DM1 physiological phenotypes, without the confounding effects of developmental defects. We crossed the 1151-Gal4 lines with transgenic lines containing 60 (UAS-CTG60) and 250 (UAS-CTG250) to represent the unaffected and DM1 mutant phenotypes. 1151-Gal4>UAS-CUGexp flies do not show skeletal muscle defects after one or two weeks of expression, as was observed in the Drosophila DM1 model using embryonic drivers. We are in the process of testing 1151-Gal4>UAS-CUGexp flies for physiological defects at 3, 4, and 5 weeks of CUGexp expression. We use tests that measure climbing velocity and flight ability in the flies to assess defects in physiological function in the flies. A subset of flies of each genotype will also be prepared for histological analysis to measure whether repeat-expressing flies exhibit muscle atrophy, as do the embryonic onset model. This study determine the time of onset of the DM1 muscle phenotypes for Drosophila DM1 model. If physiological defects are observed, this model will allow for a simplified model to perform mechanistic studies into DM1 that are unclouded by developmental defects caused by expressing repeats embryonically.

1526F **Determining the Impact Sweet Taste Receptors and Gut Microbiome have on Feeding Behavior and Glucose Metabolism in *Drosophila melanogaster*** Mikesha D Carter, Blake Riggs Biology, San Francisco State University

Diabetes mellitus is the most common metabolic disease and the 7th leading cause of death globally. One of the major factors that lead to diabetes is obesity. Obesity can be the result of addictive behaviors towards food or sugar or an altered gut microbiome. It has been proven that individuals with diabetes mellitus have dulled sweet taste reception and addictive response to sugar based on this lack of sensation. Understanding the impact that sweet taste receptors and gut microbiome have on feeding behaviors is poorly understood. We are investigating the connection between defects in sweet taste receptors and the effects on the gut microbiome. As a model organism, Drosophila are easy to manipulate genetically and can be produced with known gut bacterial species and without any gut microbiome. Drosophila have two major species of gut bacteria, Lactobacillus and Acetobacter and two primary sweet gustatory receptor neurons (GRNs) Gr64a-f and Gr5a. Here we used a Two-Choice test (feeding assay) and spectrophotometry to measure feeding preference of these flies. Also, using qPCR to measure DILP (Drosophila insulin-like peptide), a protein that is homologous to insulin and insulin-like growth factor (IGF), to measure the impact on glucose homeostasis. These results will show the effect sweet taste reception and the gut microbiome have on feeding behavior and diabetic phenotypes.

1527F **The Role of Two isoforms of Fis1 Protein in Pink1/Parkin pathway** CHUNHONG CHEN[1], Rei-Wen Liu[2] [1]National Institute of Infectious diseases and Vaccinology, [2]Institute of Bioinformatics and Systems Biology, National Chiao Tung University

Mitochondrial quality plays an important role in aging-related diseases. Mitochondria fission/fusion, and mitophagy cooperate to maintain physiological health by eliminating damaged mitochondria. Mitophagy is a process that dysfunctional mitochondria are eliminated by autophagy. Mitophagy is mainly regulated by the Pink1 (PTEN-induced kinase 1)/Parkin (E3 ubiquitin ligase) signaling pathway. Previous study showed Fission1 (Fis1) controls mitochondrial fission in yeast and Fis1

contributes to mitophagy. In this study, we are interested in whether Fis1 is involved in the Pink1/Parkin-mediated mitophagy. Accumulation of phagophore, reactive oxygen species (ROS) and ubiquitinated proteins in mitochondria were detected in the fis1 mutant. There are two major isoforms of Fis1 in fly, Fis1-LL/LS. In fis1 mutant, mitophagy marker accumulation and ROS stress were compromised by genetically expressing an isoform of Fis1-LS (Fis1-long- form short), but not by Fis1-LL (Fis1-long-form long). These results indicated that Drosophila Fis1-LS isoform functions similar to mammalian Fis1 and Fis1-LL locate on the mitochondrial contact site imply it may involve in other mitochondrial dynamics. And Fis1-LS may involve in peripheral fission. In this study, We found Fis1 protein expression is regulated by Pink1. Fis1-LS can partially rescue Pink1 mutant defect including mitochondria integrity and locomotion defect. This study may provide a new role of Fis1 in fission and mitophagy.

1528F ***Drosophila* models for investigation of mitochondrial dysfunction in gliomagenesis** Tzu-Yang Lin Institute of Cellular and Organismic Biology, Academia Sinica

Glioblastoma (GBM) is the most aggressive and deadly form of brain tumor with no cure. Prior investigations into GBM patients have demonstrated obvious distinctions in the size and shape of glioma mitochondria. These morphological irregularities may impede their function and have been associated with increased tumor malignancy (Arismendi-Morillo and Castellano-Ramirez, 2008). Additionally, functional transcriptomic analyses had unveiled significant alterations in mitochondrial function in GBM specimens (Garofano et al., 2021). However, little is known about how mitochondrial morphological alterations lead to functional deficits or vice versa. We utilized *Drosophila* models for tracking gliomagenesis from health to disease. Expansion microscopy (ExM) allows us to visualize subtle structural alterations of glial mitochondria during gliomagenesis. Our preliminary data showed that the fly glioma brain harbors many fragmented mitochondria and exhibits defective mitophagy. We also discovered the upregulation of mitochondrial fission proteins and downregulation of fusion proteins occurs in glioma brains. Further assessments of mitochondrial metabolic phenotypes and the molecular mechanisms in gliomagenesis will be presented.

Arismendi-Morillo and Castellano-Ramirez. (2008). J Electron Microsc 57, 33-39.

Garofano., et al. (2021). Nat Cancer 2, 141-156.

1529F **Transcriptional Regulation of Intestinal Stem Cell Ageing** fanila shahzad, David Doupe biosciences, Durham University

Ageing is associated with a reduction in physiological functions and increased incidence of several chronic diseases. Many tissues are constantly turned over throughout adult life and the balance of cell loss and replacement is often perturbed in ageing. The *Drosophila* intestine has emerged as an excellent model for epithelial ageing. In normal homeostasis the tissue is constantly turned over as differentiated enterocytes are lost and replaced by the proliferation of stem cells. In ageing, the stem cells become misregulated, initially overproliferating and eventually failing to maintain the tissue. This occurs in parallel to decline in epithelial barrier function and dysbiosis of the intestinal microbiota. Epigenetic changes have been described as a hallmark of ageing and we are exploring how transcriptional regulation and chromatin states change with age in the intestinal stem cells. Based on published transcriptomic data we have identified a number of transcription factors whose expression changes in ageing intestine. We have used enhancer traps to characterise the expression patterns of these transcription factors in normal homeostasis and in the ageing intestine, validating age-related changes in expression. We are currently using stem/progenitor specific knockdown and overexpression to explore their functions in normal homeostasis. Future work will use targeted DamID to identify the downstream transcriptional targets of these factors in intestinal stem cells.

1530F **The effects of Tip60 Histone Acetyltransferase on cellular degeneration and motor dysfunction in Drosophila model of Machado-Joseph Disease** Sarah E Clark[1], Hung Pham[2], John M Warrick[3] [1]Biology, University of Richmond, [2]University of Richmond, [3]Biology, Univ Richmond

Machado-Joseph Disease (MJD) is a polyglutamine (PolyQ) disease that causes lethal motor neuron degeneration resulting from a CAG repeat in the Ataxin3 (ATX3) gene. These excess CAG repeats cause the ATX3 protein to misfold, altering its function and forming aggregates (clumps of protein) in cells. The biggest aggregates form in the nucleus but can also be found elsewhere in the cell. It is believed these aggregates then trap other proteins like Histone Acetyltransferases (HATs). This may prevent acetylation of histones, reducing gene transcription, and/or the production of other proteins doing essential functions in the cell. This dysfunction is hypothesized to lead to cellular dysfunction and degeneration. Additionally, without properly functioning HAT's, it is hypothesized that motor skills in larval *Drosophila* may be negatively impacted as a result of the subsequent cellular dysfunction. HAT Tip60 is a type of histone acetyltransferase responsible for neuronal gene control and cell apoptosis. Increased levels of HAT Tip60 have been shown to reduce protein aggregation and blockages in motor axonal pathways and preventing degeneration in Alzheimer *Drosophila* models. Previous work on our model showed that the upregulation of Tip60 reduced neurodegeneration. Through immunohistochemistry and confocal microscopic analysis, we have observed reduced levels of blockages in photoreceptor axons in *Drosophila* with upregulated Tip60 protein and increased

blockages in flies with downregulated Tip60. We are currently examining larval locomotor behavior. As expected, larvae with MJD have reduced locomotor abilities. We intend to determine if co-expression of Tip60 will rescue this behavior. These results support the hypothesis that Tip 60 has a rescuing effect on cellular and motor degeneration in *Drosophila* MJD.

1531F **Modeling Dentatorubral-pallidoluysian atrophy in *Drosophila*** Matthew V Prifti, Nikhil C Patel, Kozeta Libohova, Kristin A Richardson, Sokol V Todi Pharmacology, Wayne State University

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant, progressively degenerative disorder that is predominantly distinguished by ataxia, myoclonus, epilepsy, choreoathetosis, dementia, and psychiatric disturbances. DRPLA is a member of the polyglutamine (polyQ) family of diseases, which comprises nine members and is caused by abnormal expansion of a polyQ tract repeat in specific proteins. The gene at the core of DRPLA is ATROPHIN-1 (ATN1). ATN1 is a transcriptional co-repressor that is expressed in the central nervous system and other organs. When the polyQ repeat of ATN1 is expanded beyond normal ranges, it causes neuronal toxicity and ultimately cell death. The precise molecular mechanisms underlying DRPLA are unclear. The pathogenic CAG repeat length of ATN1 ranges from 48 to 93. To assist with the understanding of the biological processes at the core of DRPLA, and with the hope of supporting the identification and development of therapeutics for this incurable disease, we recently generated isogenic *Drosophila melanogaster* transgenic lines that express full-length, human ATN1 with a normal (Q7) or pathogenic (Q88) repeat. We are in the process of characterizing these new models via longevity and motility assays and will present a full phenotypic and biochemical characterization of these new lines.

1532F **Modeling and exploring Spinal and Bulbar Muscular Atrophy in *Drosophila*.** Kristin Richardson[1], Medha Sengupta[2], Alyson Sujkowski[1], Kozeta Libohova[1], Autumn C. Harris[1], Robert J Wessells[3], Diane E Merry[2], Sokol V Todi[1] [1]Pharmacology, Wayne State University School of Medicine, [2]Thomas Jefferson University, [3]Physiology, Wayne State University School of Medicine

Spinal and bulbar muscular atrophy (SBMA) is a rare X-linked, neurodegenerative disorder resulting from pathogenic PolyGlutamine (polyQ) tract elongation within the androgen receptor (AR) protein. To add to the existing toolbox of SBMA models for studying disease pathology and therapeutics, we have generated a new model of SBMA in *Drosophila.* The newly generated lines express full-length, human AR, with either wild-type (Q20) or polyQ-expanded (Q112) AR inserted into the same “safe-harbor” site on the third chromosome. PolyQ-AR expression in adult muscle or nervous tissue leads to consistent, progressive reductions in lifespan and mobility, accompanied by aggregation of polyQ-AR protein and deterioration of the neuromuscular junction. These phenotypes are generally worsened by the addition of the AR ligand, Dihydrotestosterone. Experiments are in progress using these new lines to examine the impact of strategic mutations that will further illuminate the biology of AR protein in SBMA.

1533F **Protein L-Isoaspartyl Methyltransferase (PCMT) expression levels affect *Drosophila melanogaster* tolerance to certain bacterial infections** Maryam I Azeem[1], Maricarmen S. Carreto[2], Anđela Savić[2], Jessica Ahon Sanchez[2], Jessica Ashley Allen[1,3] [1]Columbia College of SC, [2]Roosevelt University, [3]Biological, Physical, and Health Sciences, Roosevelt University

Protein L-isoaspartyl methyltransferase (PCMT) is an evolutionarily conserved protein which initiates repair to proteins damaged by oxidative stress. PCMT is known to be important for cell survival across various cell types in different environments. Reports have shown that *Drosophila melanogaster* overexpressing *Pcmt* have an extended lifespan when raised at elevated temperatures and that male flies lacking *Pcmt* die faster during systemic *Listeria monocytogenes* infections despite a functional immune response limiting bacterial growth. Here we share data further elucidating the role of PCMT during bacterial infection.

We report that overexpression/knockdown of *Pcmt* extends/reduces the lifespan of both male and female flies infected with *Staphylococcus aureus* via septic prick. The same is true for flies infected with *Serratia marcescens* via oral infection, but not during *S. marcescens* infection via septic prick. *Pcmt* expression levels do not affect resistance to either bacterium via either infection method, as overexpressing and knockdown flies control bacterial load equally well throughout infection. Consistent with a potential role in tolerance during infection, wild type Oregon-R and Canton-S male flies induce expression of *Pcmt* upon infection with *S. marcescens.* We are currently working to determine how *Pcmt* expression levels impact non-survival measures of physiological health throughout infection and in which cell types *Pcmt* expression is important for increased tolerance to infection.

1534F **The circular RNA *circ_ATP8B* regulates ROS production and antiviral immunity** Weihong Liang[1], Wei Liu[1], Xiao-Peng Xiong[2], Jennifer W. Li[3], Jian-Liang Li[4], Ranjan J. Perera[1], Rui Zhou[5] [1]Johns Hopkins University, [2]Sanford Burnham Prebys Medical Discovery Institute, [3]Brown University, [4]National Institute of Environmental Health Sciences, [5]Johns Hopkins University School of Medicine

Circular RNAs (**circRNAs**), which are products of "head-to-tail" back-splicing events, have been discovered in diverse eukaryotes, and constitute a new addition to the non-coding and regulatory RNA collection. CircRNAs have been implicated in a wide variety of biological processes, including antibacterial innate immunity, neurodevelopment, and gene control.

To investigate the role of circRNAs in antiviral defense in *Drosophila*, we performed RNA sequencing and identified and validated of a collection of circRNAs that display significant changes in expression levels upon viral infection. We found that depletion of the pro-viral circular RNA *circ_ATP8B*, but not its linear sibling, led to a reduction in levels of viral RNA, protein and titer in cultured *Drosophila* SL2 cells upon infection by a panel of RNA viruses, including Flock House virus (**FHV**), *Drosophila* C virus (**DCV**) and Cricket paralysis virus (**CrPV**). Similarly, ubiquitous depletion of *circ_ATP8B in vivo* reduced viral load and enhanced host survival upon viral infection. Importantly, restoring *circ_ATP8B* expression in *circ_ATP8B*-depleted cells or flies suppressed the viral replication and/or host survival phenotypes. In addition, our analyses revealed that *circ_ATP8B* is a gut-enriched circRNA that is induced specifically by oral ingestion of viruses but not bacteria, and that gut-specific depletion of *circ_ATP8B* compromises viral replication in an oral infection model. Furthermore, we found that defects in viral RNA replication in *circ_ATP8B*-depleted flies correlated with elevated levels of ROS and enhanced expression of *Dual oxidase* (***Duox***), which encodes a ROS-producing enzyme. Notably, *circ_ATP8B* and Duox associate with each other, and expression of various versions of *circ_ATP8B* that are competent in binding Duox, but not a mutant *circ_ATP8B* that is incapable of binding Duox, restored physiological levels of ROS in *circ_ATP8B*-depleted cells. Lastly, we show that feeding flies with N-acetyl-L-cysteine (**NAC**), a ROS inhibitor, depletion of *Duox* or *Gaq,* a G protein required for optimal Duox activity, or overexpression of *immune-regulated catalase* (***IRC***), an enzyme that removes ROS, led to a reduction in ROS levels and suppressed the viral replication defects elicited by *circ_ATP8B* depletion. Thus, our study demonstrates that ROS acts as an antiviral agent against infection via the oral route and that *circ_ATP8B* regulates antiviral immunity, at least in part, by modulating Duox-dependent ROS production.

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1535F **Targeted genetic screen for enhancers and suppressors of lifespan in a model of Huntington's Disease in *Drosophila*** Sevinch Kamaridinova, Tadros A Hana, Sabita Basnet, Kiel Ormerod Biology, Middle Tennessee State University

Huntington's Disease (HD) is an inherited neurodegenerative disorder highlighted by a progressive breakdown in neurons leading to progressive loss of motor control. Unlike other neurodegenerative disorders, HD research has focused mostly on one gene, the: Huntingtin gene (HTT). The disease is attributable to an abnormal expansion in CAG codon repeats in the gene, causing the protein product, huntingtin protein (htt), to have an expanded region of glutamine repeats (PolyQ region) which is linked to misfolding and aggregation of the protein within nerve cells. The severity of the disease and the age of onset have been shown to correlate with the degree of expansion within the PolyQ region of htt where an increase in glutamine repeats increases the pathogenicity and reduces age of onset. A *Drosophila* model of HD was previously created in the Littleton lab, where the first Exon of human htt was altered to include either 15 or 138 glutamine repeats in the PolyQ region (htt-Q15, and htt-Q138). These transgenic lines also included an RFP-tag for fluorescent imaging. Using the UAS/Gal4 system to express these transgenes in motor neurons ($Elav^{C155}$,) we demonstrated that htt-positive aggregates accumulate in the axons and at the neuromuscular junction (NMJ) in third-instar larvae which significantly reduces the intracellular trafficking of organelles like synaptic vesicles, mitochondria, and dense core vesicles. We also repeated the observation that expression of htt-Q138 in motor neurons ($Elav^{C155}$) significantly reduces adult viability. Here we target 50 different genes associated with HD pathology or huntingtin protein function using RNA interference (RNAi) and screened for changes in adult viability. Positive hits were then examined for molecular changes, like axonal and NMJ aggregation, to elucidate putative pathways altered in HD pathology or htt biological function. The results from our genetic screen may help to identify novel therapeutic targets for treatments of HD.

1536F **Investigating the role of rare genetic variants in BMP signaling genes in neurological diseases using *Drosophila*** Haley A Dostalik[1,2], Jung-Wan Mok[1,2], Oguz Kanca[2,3], Yufeng Shen[4], Carrie Welch[4], Center for Precision Medicine Models (CPMM)[5], Michael F Wangler[1,2], Hugo J Bellen[1,2], Wendy K Chung[4,6,7], Shinya Yamamoto[1,2] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Molecular and Human Geneetics, Baylor College of Medicine, [4]Columbia University, [5]Baylor College of Medicine, [6]Boston Children's Hospital, [7]Harvard Medical School

BMP signaling is critical for development and maintaining adult tissue homeostasis. Pathogenic variants within this pathway often cause genetic diseases or cancer. Previously, rare variants in a couple genes from the canonical BMP signaling pathway have been associated with neurodevelopmental disorders (NDD) through meta-analysis and case reports. However, there have

been no functional analyses or *in-vivo* studies of these variants to verify their pathogenicity.

Recently, we identified a rare, heterozygous, recurring *de novo* missense variant in *BMPR2* in 4 patients with overlapping NDD phenotypes, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Loss-of-function (LOF) *BMPR2* variants have previously only been implicated in pulmonary arterial hypertension (PAH), which is not seen in any of these patients. Through functional studies in *Drosophila*, we have shown this variant is a gain-of-function (GOF) allele, leading to overactivation of BMP signaling. We have also identified 13 likely LOF variants in *SMAD6* associated with various NDD phenotypes, such as ASD, seizures, global developmental delay, and abnormal brain development. Previously, *SMAD6* variants have only been implicated in causing an aortic valve disease, which is not seen in the 13 affected patients. Since SMAD6 is known to function as a negative regulator of this pathway, these variants may also lead to overactivation of BMP signaling, causing clinical phenotypes that overlap with patients who carry the GOF *BMPR2* variant. In *Drosophila*, while functional studies of BMP signaling mediated by dpp and gbb have been studied in the context of neuromuscular junction development and physiology, the role of this pathway kin the central nervous system is ill defined. The goal of this ongoing study is to test the hypothesis that *BMPR2* GOF and *SMAD6* LOF variants cause overactivation of BMP signaling, leading to neurological phenotypes in humans using a *Drosophila* model. By generation of transgenic lines that allow expression of human *BMPR2* and *SMAD6*, and manipulating their fly orthologs *wit* and *Dad*, we are investigating the biological function of this conserved pathway in the larval and adult nervous system in *Drosophila*.

1537F **Functional analysis and classification of rare genetic variants in SATB2 using *Drosophila*** Hirokazu Hashimoto[1,2], Samantha L Deal[3], Oguz Kanca[1,2], Kenji Yokoi[4], Shinya Yamamoto[1,2] [1]Molecular & Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Howard Hughes Medical Institute and Chronobiology and Sleep Institute, University of Pennsylvania, [4]Nanomedicine, Houston Methodist Research Institute

SATB2-associated syndrome (SAS, a.k.a. Glass syndrome: OMIM #612313) is a rare genetic disorder with over 750 known cases worldwide. This syndrome is characterized by developmental delay, intellectual disability, and craniofacial dysmorphisms with variable expressivity and penetrance. This gene encodes for an evolutionarily conserved DNA binding protein that regulates chromatin remodeling. Because genotype-phenotype relationships in SAS have not been clearly established, we assessed the functional consequences of disease-associated variants in *SATB2* using fruit flies. After generating transgenic flies that express human *SATB2* under the control of the GAL4/UAS system, we compared the phenotypes induced by tissue-type or cell-type specific ectopic overexpression of reference or variant SATB2 *in vivo*. We studied two nonsense variants and 12 missense variants (5 variants at CUT1, 5 variants at CUT2, and 2 variants at HOX domain) previously reported in SAS patients. We found that overexpression of reference and variant human SATB2 in the wing, dorsal thorax, eye, neuron, or glia of *Drosophila melanogaster* permits the classification of disease-associated genetic variants. First, two nonsense variants behaved as strong loss-of-function (LOF) alleles *in vivo*, rather than as dominant negative alleles. Second, the early CUT1 variants behaved as a milder LOF allele compared to the nonsense variants. Last, the late CUT1 and CUT2 variants behaved primarily as gain-of-function (GOF) alleles *in vivo*.

In conclusion, some *SATB2* variants found in SAS patients behaved as LOF alleles, whereas others behaved as GOF variants. Understanding the functional consequence of each patient's variant has clinical implications because therapeutic design should be different for patients with LOF variants (e.g., gene therapy) and GOF variants (e.g., antisense oligonucleotide).

1538F **The Effects of Mutated Amyloid Peptide Expression in a Drosophila Model of Alzheimer's Disease** Kaliah Wood[1], Brett Chan[1], Aahwan Koirala[2], Olivia Nardell[2], Caroline Yu[2], Anisha Kavarthapu[2], Jorge Moran[2], Jevgenij Raskatov[3], Jeremy Lee[1] [1]Department of Molecular, Cell and Developmental Biology, University of California Santa Cruz, [2]University of California Santa Cruz, [3]Department of Chemistry and Biochemistry, University of California Santa Cruz

Alzheimer's disease (AD) is a neurodegenerative disorder that currently affects over 55 million people worldwide and is expected to affect closer to 140 million people by 2050. AD is characterized by plaques composed of amyloid beta (Aβ) peptide, which aggregates and is believed to be a major cause of neurodegeneration in AD. Recent research has demonstrated that amyloid alpha (Aα, also known as P3), a truncated form of Aβ without the first 16 amino acids, also aggregates and induces neurodegeneration. The Raskatov Lab at UCSC has identified 2 key missense mutations in Aβ, Q15E and N27D, which reduce its aggregation and cytotoxicity in vitro. We are testing the effects of expressing these 2 mutant forms of Aβ in vivo in a Drosophila model. Prior research has shown that flies expressing wild type (WT) Aβ and Aα have shorter life spans, behavioral deficits, and decreased locomotor capabilities. For this study, we transgenically express each Aβ mutation individually as well as in a double mutant. We are also working to generate flies that express WT and mutant forms of Aα.

A series of assays will be used to compare the phenotypes of WT (non-expressing) flies, flies expressing WT Aβ, and flies expressing the mutated forms of Aβ. In the longevity test, we hypothesize that WT flies will survive longest and flies pan-

neuronally expressing WT Aβ will have decreased survival. If the mutant forms of Aβ have decreased toxicity and aggregation in vivo, consistent with the in vitro results, the flies expressing mutated Aβ pan-neuronally will survive longer than WT Aβ flies. We will also perform olfactory learning assays to measure the flies' short and long-term memory. We expect that WT flies will have the best memory, and flies expressing WT Aβ will have the worst, with flies expressing mutated Aβ performing better than flies expressing WT Aβ. We will also compare the phenotypes of flies expressing the various forms of Aβ in the eyes by expressing WT and mutated forms of Aβ exclusively in the developing neurons of the eyes using a GMR-Gal4 driver. We have shown that flies expressing WT Aβ in eye neurons show defects in eye morphology, and hypothesize that expression of the mutant forms of Aβ will lead to a less severe phenotype. This research will provide essential information about structural elements of Aβ that contribute to its neurotoxicity and may guide the development of more effective therapeutics.

1539F **Tubulin and actin cytoskeletons of *Drosophila* nephrocytes and their roles in maintaining the filtration structure** Megan Delaney[1], Yunpo Zhao[2], Joseph Lee[2], Zhe Han[2] [1]Epidemiology and Human Genetics, University of Maryland-Baltimore, [2]Precision Medicine and Disease Modeling, University of Maryland-Baltimore

Drosophila nephrocyte shares striking similarities with human podocytes in the highly specificized filtration structure called Slit Diaphragm (SD). It has been established that changes in the podocyte actin cytoskeleton affect the integrity of the SD and the overall function of the glomerular filtration barrier. Nephrotic Syndrome (NS), a disease that affects 16 in 100,000 people worldwide, can be caused by variants in genes encoding cytoskeleton components. However, the main components of the nephrocyte cytoskeleton remain unclear. It is also not known whether tubulin cytoskeleton plays important roles in nephrocyte or podocyte structure and function. Using single-cell RNA-seq, my lab elucidated the genes encoding actin and tubulin cytoskeleton components for nephrocytes. We then silenced each of them using a nephrocyte Gal4 driver and the UAS-RNAi transgenic lines for these genes and identified main cytoskeleton components for nephrocytes. We found that Act5C and βTub56D are the key components for nephrocyte cytoskeleton, since silencing these genes individually will totally abolish the formation of nephrocytes. Although the knockdown of αTub84B did not result in the complete loss of nephrocytes, it does show a drastic phenotype in the SD, actin, and tubulin structures. For other actin (Act57B, Act42A, and Act87E) or tubulin (αTub84D, αTub85E, βTub97EF, and βTub60D) genes expressed in nephrocytes, we observed a variety of phenotypes, including decreased SD density, internalized SD proteins, and clustering of the actin or tubulin cytoskeleton in certain areas of nephrocytes. We also found that tubulin and actin cytoskeleton are interdependent. When tubulin cytoskeleton is disrupted, actin cytoskeleton falls apart and SD proteins accumulated with aggregated F-actin. When actin cytoskeleton is disrupted, tubulin cytoskeleton is disrupted and aggregated but without SD proteins in the aggregation. We believe that the cell uses different variants of the actin and tubulin monomers during each phase of growth, allowing for formation, development, and maintenance of the nephrocyte. In conclusion, while previous research has focused on the importance of the actin cytoskeleton in podocytes and its roles in NS, we indicated that the tubulin cytoskeleton plays a previously unknown and key role in the structure and maintenance in Drosophila nephrocytes and SD structures.

1540F **Investigating the bidirectional relationship between traumatic brain injury and sleep homeostasis in *D. melanogaster*** Rebecca N Ray, Rebecca Delventhal Biology, Lake Forest College

Traumatic brain injury (TBI) occurs when a sudden, severe impact to the head causes brain damage. This can impact short- and long-term health and homeostatic behaviors, like sleep. Recent studies have established *D. melanogaster* as a model for TBI that displays many characteristics of TBI in humans. Since sleep is a conserved behavior in nearly all animals, we decided to investigate the relationship between TBI and sleep using *D. melanogaster.* To test how TBI affects sleep over time, we used the High-Impact Trauma (HIT) device to injure wild-type flies and measured their sleep in the days and weeks post-injury. We found that at 48 hrs and 1 wk post-TBI, injured flies had more fragmented sleep than uninjured controls but progressed to sleeping more at 2 and 4 weeks. We then asked if disrupting sleep via a genetically short-sleeping fly had a detrimental effect on TBI recovery. We found that injured short-sleeping flies had a significantly higher acute mortality than injured flies with normal sleep, suggesting sleep has a protective effect on TBI recovery. We next asked what the underlying mechanism of this bidirectional relationship between TBI and sleep might be. Prior research supports the theory that sleep helps clear reactive oxygen species (ROS), protecting against oxidative stress. To investigate whether ROS mediates the relationship between sleep and TBI, we manipulated the neuronal expression of the antioxidant *SOD2* in order to change neuronal ROS levels and measured both short- and long-term TBI recovery. *SOD2* knockdown in neurons had no effect on acute mortality or lifespan within injury conditions. Interestingly, injured flies with *SOD2* overexpression in neurons had significantly higher acute mortality than injured flies with normal *SOD2* expression. Injured and uninjured flies with *SOD2* neuronal overexpression also had a decreased lifespan compared to controls with normal *SOD2* levels. This poses new questions as to how neuronal antioxidant expression affects ROS levels and oxidative stress throughout the body and if successful TBI recovery requires clearance of ROS in other tissues besides neurons. Future research manipulating ROS levels ubiquitously and in other cell types could further our understanding of ROS's role in the relationship between TBI and sleep, which could have implications in new TBI prevention or recovery strategies.

1541F **Systemic cardiac dysfunction in tumor models of *D. melanogaster*.** Shubha Gururaja Rao[1], Harpeet Singh[2], Leah Pyter[2], Priyanka Karekar[2], Sarah Seeley[1] [1]Ohio Northern University, [2]The Ohio State University

Cancer and heart diseases are the two leading mortality and morbidities in the United States. Several cancer patients undergo heart related complications resulting in their death. Cardiotoxicity due to therapeutic agents used in cancer treatment is one of the main causes of cardiac dysfunction in cancer patients. However, it is not clear if cancers systemically affect heart function prior to treatment hence making them vulnerable to cardiac abnormalities in later stages. In order to test this, our study uses different genetic models to establish if cancers indeed cause cardiac defects on their own prior to therapeutic intervention. We analyze in depth, various oncogenic pathways in *Drosophila,* such as Yki, JAK-STAT, RAS etc. and study their system effects on cardiac function. Our results indicate that oncogenes not expressed in the heart can cause cardiac dysfunction. We rescue some of these cardiac phenotypes using antioxidant treatment, with which we conclude that, systemic effects of tumorigenesis caused increased ROS affecting cardiac function. Our results also show that different oncogenic pathways have variable degrees of effects on the heart and it is important to conduct further studies to understand the systemic effects of cancers on heart function.

1542F **JAK-STAT pathway activation compromises nephrocyte function in a *Drosophila* high-fat diet model of chronic kidney disease** Yunpo Zhao, Jianli Duan, Joyce van de Leemput, Zhe Han University of Maryland

Background Obesity is a major risk factor for diabetes and chronic kidney disease, and elevated body mass index (BMI), a marker for obesity, has been associated with diabetic nephropathy and end-stage renal disease. However, our understanding of the mechanisms that link obesity to kidney disease remains incomplete.

Methods This study used a *Drosophila* model of chronic kidney disease to investigate the effect of a high-fat diet (HFD) on nephrocyte function (FITC-albumin and 10 kD Dextran uptake assays) and morphology (anti-Pyd immunochemistry; transmission electron microscopy), and to identify the underlying molecular pathway (RNAi; mutant forms; overexpression; pharmacological treatment).

Results We show that HFD-reduced absorption function of nephrocytes, the functional equivalent to human podocytes, is due to it altering the morphology of the slit diaphragm filtration structure (Pyd localization; lacuna channel distance and electron dense vacuoles). We demonstrate that HFD activates the JAK-STAT pathway in nephrocytes and that this can be initiated by increased expression and release of the adipokine, Upd2, from the fat body. Upd2 is the functional equivalent of Leptin and a ligand for the JAK-STAT pathway. Finally, we show that inhibiting the JAK-STAT pathway, either genetically or by pharmacological intervention (methotrexate), can reverse the HFD-induced nephrocyte functional deficit.

Conclusions Our study reveals the importance of the JAK-STAT signaling pathway in the adipose tissue–nephrocyte axis and its contribution to HFD-associated nephropathy. These findings open new avenues for intervention in treating diabetic nephropathy and chronic kidney disease.

1543F **The therapeutic potential of sulforaphane on the cognitive and behavioral impacts of Alzheimer's disease in *Drosophila melanogaster*** Najeeb Marun[1,2,3], Nguyen Le[4], Carmen P. Wong[2,5], Chaz Kayser[1,2], Alysia D. Vrailas-Mortimer[1,2], Emily Ho[2,5], Nathan T. Mortimer[1,2] [1]Department of Biochemistry & Biophysics, Oregon State University, [2]Linus Pauling Institute, Oregon State University, [3]Honors College, Oregon State University, [4]School of Biological Sciences, Illinois State University, [5]School of Public Health and Nutrition, Oregon State University

Alzheimer's disease (AD) is a progressive neurodegenerative disease caused by accumulation of amyloid beta (Aβ) peptides, leading to the formation of amyloid plaques. These plaques are believed to contribute to AD pathogenesis via disruption of neural connections and neurodegeneration. *Drosophila melanogaster* are valuable model organisms for the study of neurodegenerative diseases including AD. Transgenic expression of human Aβ (hAβ) in flies induces amyloid plaque formation and Alzheimer›s-like phenotypes, making flies an excellent system for investigating AD pathogenesis. We, and others, have demonstrated that hAβ expression leads to defects in memory, sleep, and locomotor activity. More specifically, we find that hAβ expressing flies perform worse in climbing assays, in which they exhibit reduced climbing speed, suggesting potential neurodegeneration affecting their locomotor ability, and in memory assays, in which they fail to alter their behavior in a choice assay following exposure to parasitoid wasps. The objective of this project is to understand the behavioral and cognitive consequences of AD in flies and investigate the potential health benefits of sulforaphane (SFN), a compound found in broccoli, as a therapeutic intervention.

To explore the therapeutic potential of SFN, broccoli seed supplements containing high amounts of SFN precursors were mixed with standard fly food. Initial feeding experiments demonstrated that flies successfully consume SFN, and we have determined

the ability of flies to survive eating food with varying concentrations of SFN. We further find that SFN is metabolized within the flies' digestive system, leading to production of previously characterized metabolites, and indicating its bio-accessibility for further testing in our hAβ expressing flies.

1544F **Investigating the role of the *Drosophila* IMD NF-κB pathway in age-dependent immunity following infection with Flock House virus** Victoria Faber[1], Josiah Cooperwood[1], Lakbira Sheffield[2], Stanislava Chtarbanova[1] [1]Biological Sciences, The University of Alabama, [2]UAB

As organisms age they become increasingly susceptible to infection and disease, including infections with viral pathogens. Although considerable progress has been made in understanding the age-related functional decline of the immune system, a complete picture of the mechanisms contributing to survival outcomes of older hosts in response to infections is still lacking. Our lab has modeled aged host-virus interactions using *Drosophila melanogaster* and the positive stranded RNA virus Flock House Virus (FHV). We have previously shown that in comparison to younger hosts, FHV infection of aged *Drosophila* contributes to decreased survival associated with impaired disease tolerance and increased expression of several genes encoding for components of the *Drosophila* immune deficiency (IMD) NF-kB pathway. This led us to hypothesize that FHV infection results in immunopathology in older hosts because of elevated IMD activation. Using RNAi, we investigated how ubiquitous knockdown (KD) of the IMD components *Relish*, *Pirk*, and *dSTING* affects survival of FHV infection with aging. Our results show that in comparison to controls, Pirk KD caused significant decrease in survival of FHV for both young and aged cohorts in both sexes. Interestingly, *dSTING* KD led to a non-significant difference in survival of FHV in young flies, but a protective effect specifically in aged females. Both young and aged *Relish* KD flies of both sexes died faster of FHV infection, possibly because of secondary bacterial infection. These data point to a causative link of overactive NF-kB pathway and survival of virus infection at older age; however additional experiments are underway to prove that this is the case. Current work focuses on carrying additional survival analysis of null mutants for both *dSTING* and *Relish*, and determining virus loads in all experimental conditions tested. Because *Relish* mutants and KD are immunocompromised and prone to acquire secondary bacterial infections as they age, we are conducting similar analysis in germ-free flies. Overall, our studies suggest a possible involvement of the IMD pathway in immunopathology after virus infection with aging and that modulation of disease tolerance responses in the older hosts could represent a target for improving the outcomes of viral infection.

1545F **Exploring the Function of a Missense Variant in *WWC3* as a Potential Candidate for a Novel Mendelian Disease using Drosophila** Jasmine O Brown[1,2,3], Shelley B Gibson[1,3], Hector R Mendez[4], Chloe Reuter[5], Shruti Marwaha[4], Devon Bonner[5], Jennefer N Kohler[5], Elijah Kravets[4], Undiagnosed Disease Network[1,4], Oguz Kanca[1,3], Michael F Wangler[1,3], Hugo Bellen[1,3,6], Euan Ashley[4], Stephen B Montgomery[4], Jonathan A Bernstein[4], Matthew T Wheeler[4], Shinya Yamamoto[1,3,6] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Post-baccalaureate Research Education Program, Baylor College of Medicine, [3]Jan and Duncan Neurological Research Institute, Texas Children's Hospital, [4]Stanford University, [5]Stanford Health Care, [6]Department of Neuroscience, Baylor College of Medicine

Hippo signaling is an evolutionarily conserved signaling pathway that is important for proper development and physiology in diverse species. In humans, dysregulation of this pathway has been linked to diverse genetic disorders as well as cancer. *WWC3* encodes one of three paralogous scaffolding proteins that acts as a positive regulator of this pathway. While *WWC3* has been studied in the context of oncogenesis, this gene has not yet been associated with a Mendelian disease. Here we present a hemizygous missense variant (p.D928N) in *WWC3* that was identified by the Undiagnosed Disease Network as a prime candidate of a rare and undiagnosed disease in a 14-year-old male. This patient presents with developmental delay, cardiac defects, myopia, recurrent pneumothoraces, apical blebs, flat feet, digital contractures, gray sclerae, soft translucent skin, and dysmorphic features. Previous studies in fruit flies (*Drosophila melanogaster*) showed that loss of function alleles of the *WWC* family ortholog, *kibra*, is recessive lethal and causes an overgrowth phenotype in mosaic animals, consistent with its role in hippo signaling in this model organism. In addition, overexpression assays in flies induced multiple scorable phenotypes during development, suggesting that this is a dosage sensitive gene. In this project, we aim to 1) assess whether the function of WWC3 and Kibra are conserved, 2) determine the functional consequence of the p.D928N variant based on rescue and overexpression experiments, and 3) explore the precise expression pattern of fly *kibra* to gain additional biological insights. So far, we have successfully created constructs with human reference or patient variant WWC3 cDNA which were confirmed by whole-plasmid sequencing. Using these constructs, we will generate transgenic flies that allow the expression of either the reference or variant human WWC3 as well as wild-type or mutant (carrying a p.D993N mutation that is analogous to patient variant) fly Kibra. Next, we will assess the capability of the reference or the variant WWC3/Kibra's to rescue the phenotype found in *kibra* mutants. We will also overexpress these transgenes in different developmental contexts to assess whether the variant functions as a loss- or gain-of-function allele. Finally, I will determine when and where fly *kibra* is expressed by assessing the expression pattern of gene- or protein-trap alleles. These results will help to elucidate the role of WWC3 and the p.D928N variant in the potential novel Mendelian disorder and will also help provide new biological insights on *WWC3*'s function in human disease.

1546F **New insights into the role of MECP2 in Rett Syndrome and Autism Spectrum Disorder through functional analysis in yeast, flies and human cell lines** Christopher Loewen[1], Jessica Schmitt[2], Barry Young[2], Seevasant Indran[2], Jie Liu[2], Eric Chen[2], Graeme McIntosh[2], Sonia Medina Giro[2], Vedanta Khan[3], Sanja Rogic[2], Jesse Chao[3], Paul Pavlidis[2], Douglas Allan[2] [1]University of British Columbia, [2]University of British Columbia, [3]University of Toronto

Gene variant discovery is becoming routine, but it remains frustratingly difficult to interpret the functional consequence or disease relevance of most identified rare variants. Experimental assays are helping to fill this interpretation gap, but there remain many roadblocks in creating assays that can interpret variant function with clinical predictive value. These include issues with assay reproducibility, scalability to hundreds of variants, and relevance to the pertinent disease mechanism. We have developed an approach that utilizes complimentary functional assays engineered in yeast flies and human cell lines to address these problems. We report on our efforts towards characterizing variants found in Rett syndrome and ASD in the MECP2 gene. In yeast, we exploited the slow growth phenotype resulting from MECP2 overexpression as a functional assay and tested 46 variants. Benign variants had little effect as expected, whereas pathogenic variants located in the MBD (the DNA binding domain) had large effects, indicating the disease-relevance of this assay. Genetic interaction screens for MECP2 showed enrichment for nuclear-related functions implicating these as the relevant disease pathways. MECP2 overexpression in the developing fly wing tissue caused generation of ectopic wing vein tissue in the wing. This was suppressed by heterozygosity for Asx, a fly co-repressor, indicating that MECP2 phenotypes arise through excess transcriptional repression. Benign and pathogenic behaved as normal and loss of function variants as expected. We then found that 4/7 ASD MECP2 variants are likely loss of function variants, and 3 are likely benign. Using HEK293 cells we assessed protein stability and cellular localization of all variants. Together our results reclassify ASD variants of uncertain significance as either likely benign or pathogenic.

1547F **Role of SETDB1 in hematopoiesis: A hold on expression of *HOX* genes** Indira Paddibhatla[1,2], Dushyant Kumar Gautam[3], Sreevani Palat[4] [1]Cardiology, Johns Hopkins University, School of Medicine, [2]Biochemistry, University of Hyderabad, [3]Biochemistry, School of Medicine, University of Hyderabad, [4]Indian Institute of Science Education and Research (IISER) Tirupati.

Proper regulation of genome by the epigenetic mechanisms is crucial for an error-free and steady growth of cells. Epigenetic mechanisms are heritable alterations affecting physical or chemical organization of the chromatin. *SETDB1* is a gene that encodes a histone methylase and mediates epigenetic modification; methylation of histone-3 occurs at lysine-9 silencing its target genes. In various cancers (like melanoma, breast cancer, hepatocellular cancer, lung cancer) *SETDB1*expression is elevated. We have discovered that in *Drosophila* SETDB1 is required for hematopoiesis. Mutants with loss of SETDB1 function (*dSETDB1*$^{-/-}$) exhibit hematopoietic overgrowth of the lymph gland (hematopoietic organ) decreased crystal cells along with an increase in the lamellocyte population that form blood-tumors. While dSETDB1 is essential for normal hematopoiesis, the molecular mechanism by which SETDB1 functions is poorly understood. We have recently identified that *HOX* genes as likely targets of SETDB1. We used microarray studies to determine differentially expressed genes in third instar mutant (*dSETDB1*$^{-/-}$) larvae relative to their heterozygote siblings. Microarray results revealed 653 genes to be down-regulated and 598 genes to be up-regulated. Of these, some de-regulated genes were associated with development. After validation by qRT-PCR, we chose to focus on the four *HOX* genes (*Abd-a*, *Abd-b*, *Dfd* and *Ubx*) because their ectopic expression using the immune cells' (blood cells and/or the fat body) specific GAL4s (*Collagen-Gal4*and *Hemese-Gal4*) in *Drosophila* resulted in tumor formation along with infiltration of the fat body tissue. Furthermore, *Collagen>Abd-A* larvae with overexpression of *SETDB1* expression (*UAS-SETDB1*) did not show any melanotic tumors in the circulating hemolymph. Lastly, we have discovered that the penetrance of blood tumors formed in *Collagen>Abd-A*larvae was decreased upon azacytidine (anti-leukemia drug) treatment along with increased expression of *SETDB1* and restoration of cell cycle regulators' expression (*Cyclin A*, *Cyclin D* and *P21*). Based on these observations, we speculate that SETDB1 is involved in the regulation of *HOX* genes in *Drosophila* blood cells.

1548F ***Drosophila* Modelling Reclassifies VoUS in *EED* and *SUZ12*: Relevance for Clinical Interpretation and Rare Variant Burden Testing in Population Cohorts** Douglas W Allan[1], Sonia Medina Giro[2], Sharri Cyrus[2], Jie Liu[2], William Gibson[2] [1]Cellular and Physiological Sciences, University of British Columbia, [2]University of British Columbia

Polycomb Repressive Complex 2 is an epigenetic reader and writer that methylates lysine 27 of histone H3 (H3K27), thereby assisting in chromatin condensation and transcriptional silencing. Rare germline partial loss of function (LoF) variants in core member EED cause Cohen-Gibson Syndrome, whereas pLoF variants in EZH2 cause Weaver syndrome and pLoF variants SUZ12 cause Imagawa-Matsumoto syndrome. Constitutional variants in the PRC2 "core" proteins are thus implicated in overgrowth and intellectual disability syndromes, whereas somatic variants are implicated in a variety of cancers.

GnomAD v4.0 reports that rare coding VoUS in PRC2 core proteins are, collectively, relatively common among the "general population." Summation of the gnomAD-derived minor allele frequencies for all VoUS, LP and P variants (excluding Benign and

Likely Benign variants) catalogued rare PRC2 variants in 1.3% of chromosomes, or 2.6% of the gnomAD population. The signal is largely driven by rare SUZ12 variants. Variants in these genes may represent an underappreciated risk factor for common, complex diseases.

In order to classify VoUS in patients with neurodevelopmental disorders, and to power future Rare Variant Burden tests in population cohorts, we developed a *Drosophila* mimetic approach: an *in vivo* assay that builds human-derived variants in *EED* or SUZ12 into its fly orthologue *esc, or Su(z)12*. We used CRIMIC technologies in both cases to replace each gene with a cDNA for the mimetic variant gene. To create assays of variant function with clinical predictive value, we calibrated assays with known benign and pathogenic variants, and examined phenotypes of pathogenic variants, benign variants and VoUS relevant to the known function of the PRC2 complex in Drosophila.

We report that our fly mimetic approach accurately assesses the loss of function of pathogenic variants and the 'normal' function of benign variants. This successful calibration of functional assays allows us to assess and re-classify VoUS as likely benign or likely pathogenic. We are proceeding to study and reclassify multiple EED and SUZ12 VoUS from gnomAD and other cohorts, and are building similar fly assays for EZH2.

1549F **Dissecting the cell-cell communication between senescent cells and the surrounding normal cells *in vivo*** Takao Ito[1], Tatsushi Igaki[2], Lucy Erin O'Brien[3] [1]Stanford University, [2]Graduate School of Biostudies, Kyoto University, [3]Department of Molecular & Cellular Physiology, Stanford University

Cellular senescence, a state of irreversible cell-cycle arrest, has been attracting much attention as the leading cause of organismal aging. Senescent cells accumulating in tissues during aging are considered to cause or contribute to aging phenotypes and aging-associated diseases. However, the underlying mechanisms still remain unclear: little is known about how senescent cells affect their surrounding cells and tissues, and in the first place, how senescent cells accumulate *in vivo* is unknown. Here, using *Drosophila melanogaster* as a model, we show that senescent cells promote cell mitosis in their surrounding normal cells in the intestinal epithelium of the young adult fly. Using the MARCM technique, we induced mosaic clones expressing the ETS transcriptional activator Pointed, which was identified as a master regulator of cellular senescence in the fly larval epithelial tissue (Ito and Igaki, 2021, *Sci Signal*), in the young adult intestinal epithelium. We found that Pointed clones induced in the tissue are positive for various cellular senescence markers. Thus, this approach allows us to explore the cell-cell communication between senescent epithelial cells and the surrounding normal epithelial cells *in vivo*. Using this method, we discovered that Pointed clones strongly increase the mitotic cell number in their surrounding normal cells in the young intestine. In addition, we found that these surrounding cells are positive for Delta (a marker for intestinal stem cell) and Erk phosphorylation. Erk signaling is known to play a key role in activating stem cell division (Liang *et al*, 2017, *Nature*). These observations suggest that senescent cells could open up the proliferative potential of their surrounding stem cells via elevation of Erk signaling. On the other hand, interestingly, we found that Pointed clones induced in the young intestine are eliminated from the epithelium over time. We will further discuss about the underlying mechanisms regulating these cell-cell communications *in vivo*.

1550F **Genetic diversity of Collaborative Cross mice reveals highly variable antibody response to seasonal influenza vaccine** Lucie Dupuis[1], Cécile Apert[2], Juliette Meyer[3], Pascal Campagne[3], Pascal Blanc[4], Geneviève Renauld-Mongénie[5], Daniel Larocque[6], Sylvie van der Werf[7], Gérard Eberl[8], Xavier Montagutelli[9] [1]Mouse Genetics Laboratory, Institut Pasteur, Paris, [2]Micrenvironment and Immunity, Institut Pasteur, [3]Bioinformatics and Biostatistics Hub, Institut Pasteur, [4]Global Immunology Group, Sanofi, [5]Global Antigen Design Group, Sanofi, [6]Innovation and Emerging Science Group, Sanofi, [7]Molecular Genetics of RNA viruses Unit, Institut Pasteur, [8]Microenvironment and Immunity, Institut Pasteur, [9]Mouse Genetics Laboratory, Institut Pasteur

Vaccination is one of the most effective interventions to protect against infectious diseases. Vaccines have been developed for a variety of microorganisms. Depending on the desired immune response targeted, vaccines may present antigens in different forms (e.g. attenuated or inactivated organism, purified antigen, nucleic acid encoding antigen) in the presence or absence of adjuvants aimed at enhancing immune responses. One of the most used vaccines is the seasonal influenza vaccine (IV) which composition is adapted every year to sample the most circulating influenza strains. However, induction of protective, neutralizing antibodies to this inactivated, unadjuvanted vaccine, is variable between individuals and decreases with age. Multiple host-related factors contribute to this incomplete efficacy, and studies in humans are complicated by the heterogenous history of previous exposure to influenza virus. To identify genetic factors influencing antibody response and which may contribute to the vaccine efficacy, we immunized mice from 39 CC strains with a human tetravalent IV and analyzed their antibody response against influenza H1N1 virus at several time points. CC mice showed large heritable variations in the magnitude and kinetics of antibody response, as well as in the distribution of IgG subclasses. Cytokine levels before immunization and one day after boost were also variable between strains, and random forest analysis identified patterns

associated with high or low antibody responses. These results show that CC mice, which exhibit the same variability of humoral response to IV as humans, could be a useful platform for evaluating vaccine efficacy and allow the identification of genetic variants and biomarkers associated with diverse antibody responses.

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1551F **RESF1 is a tandem G4-associated tumor suppressor in triple negative breast cancer** Megan Majocha[1,2], Devin Jackson[1], Ngoc-Han Ha[1], Kent Hunter[1] [1]National Cancer Institute, [2]Georgetown University

In the US, women diagnosed with localized breast cancer have a 5-year survival rate of approximately 99% but drops to 30% when women develop metastases. Therefore, a better understanding of metastatic biology is necessary to prevent or treat metastases. To accomplish this, we have used the highly metastatic genetically engineered mouse model (GEMM) MMTV-PyMT to identify inherited factors that influence metastasis. Genetic mapping analysis was performed in a backcross between the MMTV-PyMT model and the MOLF/EiJ mouse strain to identify genes associated with tumor burden, latency, and metastasis. A significant peak on the distal chromosome 6 was observed and several putative metastasis susceptibility genes, including *Resf1*, were identified that predict distant metastasis free survival in ER-negative breast cancer. RESF1 is a large, unstructured protein whose intron-exon structure is conserved across species, but the amino acid sequence is not. Using a CRISPR-mediated knockout mouse model, as well as a genetrap model, of RESF1 depletion, crossed to the PyMT GEMM, our lab demonstrated that *Resf1* loss is associated with increased lung metastasis, consistent with patient data. In addition, 50% reduction in RESF1 resulted in a significant increase in tumor growth and a shortened overall survival time. Furthermore, analysis of matched tail and primary tissue from the *Resf1* heterozygous and PyMT cross revealed loss of the wildtype copy, consistent with *Resf1* being a tumor suppressor at the primary site. RNA-seq data, however, showed that *Resf1* is more highly expressed in metastases compared to primary tumors, suggesting that Resf1 has opposing roles in the primary and secondary site. To study the underlying mechanism of RESF1 in metastasis, bioinformatic analyses of metastatic cell lines and mouse tumors were performed. Genomic analysis suggests that RESF1 binds to tandem G4-quadruplex DNA structures, which are alternative knot-like structures that can form in DNA with high GC content resulting in altered transcription or DNA replication. Currently, our experiments are focused on investigating this novel mechanism of RESF1 in ER-negative breast cancer metastasis.

1552F **Identification of Homeostatic Predictors of Severe Infection with Machine Learning Methods** Kalika Kamat[1,2], Brea Hampton Brown[1], Jessica Graham[3], Elizabeth Anderson[1,4], Sharon Taft-Benz[1], Tim Bell[1], Pablo Hock[1], Sarah R Leist[5], Colton L Linnertz[1], Emily Madden[6], Kenneth Plante[1], Sanjay Sarkar[1], Alexandra Schaefer[5], Ginger D Shaw[1], Victoria K Baxter[4,7], Jennifer Lund[3], Fernando Pardo Manuel de Villena[1,8], Ralph S Baric[6], Mark T Heise[1,6,8], Martin T Ferris[1] [1]Department of Genetics, University of North Carolina - Chapel Hill, [2]Bioinformatics and Computational Biology Curriculum, University of North Carolina - Chapel Hill, [3]Fred Hutchinson Cancer Research Center, [4]Department of Comparative Medicine, University of North Carolina - Chapel Hill, [5]Department of Epidemiology, University of North Carolina - Chapel Hill, [6]Department of Microbiology and Immunology, University of North Carolina - Chapel Hill, [7]Department of Pathology and Laboratory Medicine, University of North Carolina - Chapel Hill, [8]Lineberger Cancer Center, University of North Carolina - Chapel Hill

Infectious diseases manifest with differing severity in individuals due to a number of underlying factors including genetics, age, gender, environmental exposures and overall health of the immune system. Therefore, predicting disease outcomes prior to infection is challenging due to limitations in our ability to study any of these factors in isolation in the human population. One potential approach to better understanding how genetic variation affects the ability to predict different disease outcomes is to utilize mouse models that incorporate genetic diversity. Here, we utilize the Collaborative Cross (CC) genetic reference panel to assess whether aspects of steady state (homeostatic) transcription and immune cell composition can be used to predict disease outcomes to viral (SARS-CoV-2, chikungunya) infections. Harnessing the power of machine learning, we phrased this task as a classification problem for separating susceptible mice from resistant ones based solely on measurements taken prior to infection. We trained algorithms including random forest, support vector machines, logistic regression and neural networks to identify resistant mouse strains from a mixed group in our training set. We then assessed accuracy in a separate test dataset, and found that depending on the methodology, we could achieve accuracy between 50 and 78%. Our preliminary results with chikungunya infection data show highest accuracy and replicability with neural networks (NN). We further were able to compute SHAP values from these NN classifiers, and can point to important predictors of disease outcome across genetic backgrounds.

1553F **Unraveling the role of mitochondrial and NAD^+ metabolism in acute and chronic kidney disease through a cross of resistant and sensitive mouse strains** Jean-David Morel SV, EPFL

Acute kidney injury (AKI) and chronic kidney disease (CKD) are global health issues steadily rising in incidence and prevalence. Animal models on a single genetic background have so far failed to recapitulate the clinical presentation of human nephropathies. We implemented two models of kidney injury in 7 highly diverse mouse strains: (1) the AKI-to-CKD transition over 6 weeks after folic acid (FA) exposure, and (2) kidney alterations upon a prolonged feeding with western diet and housing at thermoneutrality (WD/TN). We measured plasma and urine parameters, as well as renal histopathology and mRNA expression. We observed an extensive strain-specific response, ranging from complete resistance of the CAST/EiJ mouse strain to high sensitivity to FA-induced kidney damage in the PWK/PhJ strain. In susceptible strains, AKI is accompanied by the induction of the mitochondrial stress response (MSR) and the attenuation of NAD^+ synthesis pathways. This is associated with delayed healing and a prolonged inflammatory and adaptive immune response 6 weeks after insult, heralding a transition to CKD. In the WD/TN model, the CAST/EiJ strain was again remarkably resistant to kidney damage, while kidney histology indicated tubular damage and immune infiltration in other strains. Through a thorough comparison between the transcriptomic response in mouse and human disease, we showed that critical metabolic gene alterations are shared across species and highlighted the PWK/PhJ strain as an emergent model of kidney disease and the CAST/EiJ strain as exceptionally resistant to both injury and metabolic challenge of the kidney. In order to map specific genes responsible for resistance or susceptibility to kidney disease, we performed a second generation (F2), 4-way cross between the C57BL/6J, CAST/EiJ, 129/SvlmJ and PWK/PhJ strains, and submitted the F2 mice to WD/TN treatment over 20 weeks. Across 500 F2 mice, we observe a continuum of responses of body weight gain and obesity, markers of liver disease, or of kidney disease, enabling precise gene mapping, which will be presented.

1554F **The Efficiency of Arsenic Metabolism Depends on Genetic Background in Mice Carrying the Same Humanized *AS3MT* Gene** Timothy A Bell[1], Joseph Farrington[1], Pablo Hock[1], Ginger D Shaw[2], Paul Cotney[2], James G Xenakis[2], Christelle Douillet[2], Peter H Cable[2], Matt W Blanchard[2], John S Sigmon[2], Chidima Ahulamibe[2], Joyce Woo[2], Tianyi Liu[2], Qing Shi[2], Fei Zou[2], Rebecca C Fry[2], Miroslav Stýblo[2], Fernando Pardo-Manuel de Villena[2] [1]Genetics, UNC-CH, [2]UNC-CH

In mice and humans, the arsenite methyl-transferase gene (*AS3MT*) encodes for the key enzyme responsible for metabolizing/detoxifying arsenic. Humans and mice have a single copy of this gene, but the murine version is significantly more effective in converting inorganic arsenic (iAs) into the less toxic dimethyl-arsenic (DMA). Thus, although laboratory mice have been used for decades to model the effects of arsenic exposure, the ability to translate any finding to humans remains unclear. In 2020, we and our UNC collaborators, generated a humanized mouse by replacing the entire mouse *As3mt* gene and its regulatory sequences with the homologous human sequence and demonstrated that these mice metabolize iAs in human-like fashion. As a resource to test whether genetic background has any effect on arsenic metabolism or in arsenic mediated disease phenotypes, we used these humanized animals and Collaborative Cross (CC) mice to generate multiple congenic lines carrying identical human *AS3MT* genes on diverse genetic backgrounds. To test the hypothesis that genetic background influences total arsenic (tAs), and the percent of iAs (% iAs), methyl-arsenic (% MAs), and dimethyl-arsenic (% DMAs) in excreted urine, we exposed 4 humanized congenic strains to three doses of arsenic: 0.1, 1, and 5 ppm, in drinking water in addition to controls drinking deionized water. We measured iA, MA, and DMA and calculated tAs. Urine was collected at three timepoints during the experiment, pre-exposure, midpoint, and the end of the multi-week of exposure. We performed these experiments on 12 replicates of both male and female mice from each congenic line. As expected, the levels of tAs in urine increases as the dosage increases across strains and sexes. However, the tAs excreted in the urine varies significantly between for a given dose (this is true for 1ppm and 5ppm exposures). Specifically, humanized B6 excrete significantly more (3x) than humanized mice in the CC003 background. Strain differences are also observed for arsenic composition. Finally, there is evidence for sex and diet effects. We conclude that despite the important role that *AS3MT* plays, there are additional genetic factors that influence arsenic metabolism in laboratory mice. The humanized congenic strains can be used to develop better models of exposure and identify these additional genetic factors.

1555F **Deficiencies in the mitochondrial associated membrane (MAM) component FAM105A (now OTULINL) disrupt innate immune signaling and cause fibrostenotic inflammatory bowel disease (IBD) in mice and very early onset IBD (VEO-IBD) in humans** Brooke A. Green[1,2], Anjali Thomas[1,2], Rieko Niibori[1], Amber-Anne Mullin[1,2], Neil Warner[3], Aleixo Muise[3,4,5,6], Mahmoud El-Maklizi[3,7], Miki Gams[3,7], Cynthia Guidos[3,7], Sabine P. Cordes[1,2] [1]Lunenfeld-Tanenbaum Research Institute, [2]Department of Molecular Genetics, University of Toronto, [3]The Hospital for Sick Children, [4]Department of Biochemistry, University of Toronto, [5]Department of Pediatrics, University of Toronto, [6]Insitute of Medical Sciences, University of Toronto, [7]Department of Immunology, University of Toronto

Inflammatory bowel disease (IBD) is a debilitating inflammatory intestinal condition that can involve fibrosis and strictures, which are especially frequent in Crohn's Disease (CD) and in very early onset IBD (VEO-IBD). The paucity of mouse models that recapitulate fibrostenotic IBD has limited research into identifying cell populations, such as different immune cell types, that drive intestinal fibrosis and could be targets for anti-fibrotic therapies.

A key hub for integrating innate immune signals are the contacts between mitochondria and the endoplasmic reticulum (ER) called mitochondria-associated membranes (MAMs). Disrupting immune signaling at MAMs can cause IBD and increase risk for intestinal fibrosis; for example, variants in the cytosolic bacterial immune sensor Nucleotide-Binding Oligomerization Domain Containing 2 (NOD2), which activates signaling at MAMs, confer the highest risks for ileal CD with strictures.

The Ovarian Tumor (OTU) domain protein OTULINL, unlike other OTU domain proteins, does not bind or cleave ubiquitin chains and contains a transmembrane domain that tethers it to MAMs. We have generated an *Otulinl* knockout mouseline that, excitingly, shows inflammation and fibrosis in the small intestine, recapitulating the pathology of CD. Highlighting its importance in human IBD, we identified multiple *OTULINL* variants unique to VEO-IBD patients at the Hospital for Sick Children in Toronto.

Previous analyses of NOD2-deficient CD patient tissues have led to the untested hypothesis that disrupting innate immune signaling solely in immune cells can drive fibrostenotic IBD. Here, using adoptive transfer experiments we show that immune cells drive the intestinal inflammation and fibrosis seen upon *Otulunl* loss. Moreover, in luciferase assays, OTULINL suppresses *IFNB1* transcription driven by the Mitochondrial Antiviral Signaling Protein (MAVS), which oligomerizes at MAMs and is promoted by NOD2 signalling. Strikingly, some of the VEO-IBD variants heighten this suppression and one variant enhances NOD2-driven NF-κB activity and alters subcellular NOD2 localization. Taken together, our findings suggest that immune cells drive NOD2-deficient fibrostenotic IBD and that OTULINL regulates MAM-dependent inflammatory responses.

The *Otulinl* knockout mouse presents a novel model of fibrostenotic IBD that could be harnessed for determining the cellular and molecular drivers of intestinal fibrosis which could open avenues towards its arrest or reversal.

1556F **Understanding the Mechanisms Mediating Gene-Environment Interactions in Congenital Heart Defects** Irene Zohn, Irene Zohn Center for Genetic Medicine, Children›s National Hospital

The developing embryo has the remarkable ability to buffer genetic and environmental insults to ensure normal development, but congenital anomalies result when these insults exceed a disease threshold. We aim to elucidate how these buffering systems mediate the interplay between genes and the environment and if their disruption contributes to structural birth defects in genetic syndromes. Developmental robustness, or the ability to resist perturbations, can potentially be achieved through miRNA-mediated negative feedback loops to buffer insults. However, it remains known whether miRNA-mediated buffering mechanisms have any role in birth defects. Maternal diet is an important environmental factor that can influence the development of congenital anomalies. For instance, intake of either too much or too little vitamin A can result in congenital heart defects. Moreover, pregnant women may ingest varying amounts of vitamin A, depending on diet and supplement usage, and the embryo must buffer these variations to ensure normal cardiac development. A precise gradient of retinoic acid, established by feedback loops involving retinoic acid synthesis and degradation enzymes, is necessary for the proper specification and development of cardiac progenitors. Our studies demonstrate that genetic mutations can interact with the maternal diet to alter the establishment of this retinoic acid gradient and the development of cardiac progenitors. For instance, 22q11.2 deletion syndrome (22q11DS) is associated with variable aortic arch and outflow tract defects in humans. It is well accepted that the variability in 22q11DS is due to the influence of genetic and environmental factors, but the identity of these factors and how they interact remains unknown. Utilizing a mouse model of 22q11DS, we demonstrate that variability may be due to impaired buffering of retinoid signaling. We find that a significant proportion of 22q11DS embryos from dams fed normal levels of vitamin A exhibit aortic arch but not outflow tract defects. But, when dams are fed slightly more vitamin A, 22q11DS embryos, but not their wild-type siblings, exhibit both aortic arch and outflow tract defects. Changes in buffering of retinoid signaling, miRNAs, and cardiac progenitor specification and development precede these malformations. These findings reveal a new mechanism through which genetic mutations and maternal diet changes can influence phenotypic variability in genetic syndromes.

1557F **A Machine Learning Approach to Predicting Tumor Status through Telomere Length Variation (TLV) Analysis** Priyanshi Shah, Arun Sethuraman Biology, San Diego State University

Telomeres, the protective caps at the ends of chromosomes, consist of a repetitive nucleotide sequence (TTAGGG) in humans and play a crucial role in chromosomal replication. With each cell division, telomeres shorten due to the end-replication problem. Telomere shortening is therefore associated with age-related diseases, early mortality, and has implications for cancer biology, particularly in the context of cellular senescence and tumor progression. Our preliminary analyses of Telomere Length Variation (TLV) in the 1000 Genomes Project data indicate significant variation in TLV among human populations, between sexes, and age classes. A recent study (Burren et al., 2023) also performed a large scale estimation of telomere length variation across the UK BioBank data and identified several associated rare variants that correlate with myeloid cancer precursors. Here we use data from The Cancer Genome Atlas (TCGA) in congruence with variants identified by Burren et al.,

2023 to achieve three primary objectives: 1. Quantify telomere length variation (TLV) across different cancer types and tissues. 2. Identify genetic variants associated with telomere length variation, spanning both cancer and non-cancer genomes. 3. Utilize estimated telomere lengths from the whole-genome sequencing (WGS) data, rare variant data, and phenotypic data available on TCGA to train a supervised machine learning model that predicts tumor status (cancer versus non-cancer).

1558F **Functional characterization of a pancreatic cancer GWAS signal at the chr13q12.2/*PLUT*/*PDX1* locus** Trevor A Christensen[1], Erin Char[2], Daina Eiser[2], Michael Mobaraki[2], Irene Collins[2], Jun Zhong[2], Aidan O'Brien[2], Gauri Prasad[2,3], Katelyn Connelly[2], Jason Hoskins[2], Laufey Amundadottir[2] [1]National Cancer Institute, National Institutes of Health, [2]Laboratory of Translational Genomics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, [3]2Laboratory of Receptor Biology and Gene Expression, Center for Cancer Research, National Cancer Institute, National Institutes of Health

Genome-wide association studies (GWAS) searching for common genetic variants associated with increased risk of pancreatic cancer have identified a prominent signal at chr13q12.2 overlapping the promoter region of *PDX1*, encoding a transcription factor involved in pancreas development, and the homeostasis lncRNA gene *PLUT*, which mediates *PDX1* expression. Fine mapping, epigenomic annotation, and experimental validation of allele-specific regulatory activity together implicated rs9581943 (P=1.21x10^{-12}, OR=1.16) as the most likely functional variant driving this association signal. Analysis of expression quantitative trait locus (eQTL) from the Genotype-Tissue Expression (GTEx) project identified a significant association between rs9581943 genotype and *PDX1* expression in the pancreas (P=2.5x10^{-6}), suggesting *PDX1* as a likely mediator of the association. To assess the phenotypic effects of altered *PDX1* expression, doxycycline-inducible *PDX1* overexpression models were generated in pancreatic cancer cell lines. Increasing *PDX1* expression resulted in reduced cell proliferation and an increased proportion of cells entering apoptosis. Differential expression analysis of time-course RNA-seq data found that several pathways linked to immune system activity, inflammation, diabetes, and other cancer-related pathways were affected. While allele-specific protein binding at rs9581943 has been observed by electromobility shift assays (EMSA), efforts to identify the responsible transcription factor(s) continue. To directly assess its allele-specific effects on gene expression, all three diploid rs9581943 genotypes (GG, GA, AA) were engineered from heterozygous pancreatic cancer cell lines using CRISPR/Cas9 nuclease single-base editing. After editing and clonal selection, *PDX1* expression was measured across all three genotypes and found to negatively correlate with copies of the risk allele, consistent with the eQTL results. Taken altogether, these data implicate rs9581943 as a likely mediator of functional pancreatic cancer susceptibility by modulating *PDX1* expression.

1559F **Phylogenetic Analysis of HIV Antiretroviral Drug Resistance Evolution in Sub-Saharan Africa** Pleuni S. Pennings, Takudzwa Chirenje Biology, San Francisco State University

Out of the estimated 39 million people worldwide living with HIV, 29 million are receiving antiretroviral drugs (ARVs). However, HIV drug resistance poses a formidable challenge to the effectiveness of antiretroviral drugs (ARVs) in HIV treatment. Transmitted drug resistance (when resistance evolves in one host and the resistant strain is transmitted to one or more other hosts) is seen at high levels in Sub-Saharan Africa. Understanding the evolution and transmission dynamics of HIV drug resistance mutations is imperative in addressing this growing concern. This study is directed towards investigating the evolution of resistance in Sub-Saharan Africa, given the region's limited treatment options and high HIV infection rates, which contribute to the complexity of HIV drug resistance. Using phylogenetic tools, an analysis of well-known drug resistance mutations in publicly available HIV sequences will be conducted. The primary goal is to determine the roles of transmitted drug resistance by constructing a comprehensive phylogenetic tree and then quantifying cluster sizes of drug resistance in the tree and analyzing the transmission patterns across distinct classes of HIV drugs. Anticipated outcomes include the identification of genetically related clusters of drug-resistant strains and inferring the distribution of cluster sizes, hypothesized to vary due to differences in the fitness costs of drug resistance mutations which influence their transmissibility. This research is expected to significantly contribute to the understanding of the transmission and evolution of drug resistance in HIV. The findings hold the potential to inform the development of strategies aimed at mitigating the transmission of drug resistance, consequently reducing the prevalence of drug resistance in HIV patients and preventing unnecessary fatalities. The specific aims of this study involve acquiring 40,000 HIV sequences from Sub-Saharan Africa with associated metadata to construct dated phylogenetic trees (Aim 1) and quantifying and comparing cluster size distributions for 10 important resistance mutations that confer resistance to specific classes of HIV drugs (Aim 2). This will result in an increased understanding of the roles of de novo evolution and transmission for important HIV drug resistance mutations. By addressing critical gaps in knowledge concerning HIV drug resistance evolution and transmission, this research attempts to provide valuable insights and strategies to combat the transmission of drug resistance, thereby improving the overall outcomes for individuals living with HIV.

1560F **circRNA Transcriptomic Associations with Parkinson's Disease Severity and Genetic Mutations** Sayan Biswas, Arti Jajoo, Sebastian Kadener Biology, Brandeis University

Parkinson's Disease (PD) is the second most common neurodegenerative disorder worldwide. There is an unmet need in PD diagnosis to characterize novel biomarkers that arise from molecular dysregulation in the brain due to the disease. circRNAs, a non-canonically spliced form of pre-mRNAs, accumulate with age in the brain and are enriched in neurons. circRNAs as promising biomarkers due to their unusual stability, diversity, and abundance in the brain, and detectability in minimally-invasive fluids like blood. Our study investigates the circRNA transcriptome in a cohort of over 4000 human blood samples to understand its relationship with Parkinson's Disease (PD). This is, to the best of our knowledge the largest population circRNA study until now. We identified more than 10,000,000 circRNA types in this population. Importantly, we found that most of them are expressed in only few individuals, demonstrating that most circRNAs are likely splicing noise. However, we found thousands of cicNRAS that are robustly expressed across the populations. Our analysis identified a subset of circRNAs with consistent expression patterns, potentially linked to biological relevance in PD. We performed differential expression analysis and found that circRNAs are globally downregulated in PD patients compared to controls. In longitudinal comparisons, we identified several circRNAs with consistent downregulation across PD progression visits, suggesting their potential as indicators of disease trajectory. Furthermore, we observed pronounced circRNA dysregulation in genetically determined PD cases (GBA+, LRRK2+, SNCA+ mutations) compared to idiopathic PD, reflecting the distinct influence of genetic factors on the circRNA transcriptome. By correlating circRNA expression with Unified Parkinson's Disease Rating Scale (UPDRS) scores, we uncovered strong positive correlations with scores I & IV in idiopathic PD, which were absent in genetic PD cases. Our findings highlight the value of circRNA profiling in understanding PD's molecular underpinnings and suggest circRNAs as potential biomarkers for PD severity and progression, with implications for both idiopathic and genetic forms of the disease. Moreover, we are performing additional population analysis in order to determine whether circRNAs correlate with biomark other physiological parameters.

1561F **Concurrent Evolution of Antiaging Gene Duplications and Cellular Phenotypes in Long-Lived Turtles** Stephanie E Bulls[1], Scott Glaberman[2], Juan M Vazquez[3], Ylenia Chiari[1], Vincent Lynch[4] [1]Biology, George Mason University, [2]Environmental Science and Policy, George Mason University, [3]Biology, University of California-Berkeley, [4]Biological Sciences, University at Buffalo

There are many costs associated with increased body size and longevity in animals, including the accumulation of genotoxic and cytotoxic damage that comes with having more cells and living longer. Yet, some species have overcome these barriers and have evolved remarkably large body sizes and long lifespans, sometimes within a narrow window of evolutionary time. Here, we demonstrate through phylogenetic comparative analysis that multiple turtle lineages, including Galapagos giant tortoises, concurrently evolved large bodies, long lifespans, and reduced cancer risk. We also show through comparative genomic analysis that Galapagos giant tortoises have gene duplications related to longevity and tumor suppression. To examine the molecular basis underlying increased body size and lifespan in turtles, we treated cell lines from multiple species, including Galapagos giant tortoises, with drugs that induce different types of cytotoxic stress. Our results indicate that turtle cells, in general, are resistant to oxidative stress related to aging, whereas Galapagos giant tortoise cells, specifically, are sensitive to endoplasmic reticulum stress, which may give this species an ability to mitigate the effects of cellular stress associated with increased body size and longevity.

1562F **Genetic background modifies metabolic related outcomes after inorganic arsenic exposure in humanized *As3mt* mice.** Ginger D Shaw[1], Timothy A Bell[1], Joe Farrington[1], Pablo Hock[1], Chidima Ahulamibe[1], Matt Blanchard[1], Paul Cotney[1], David Truong Ngyen[1], John Sebastian Sigmon[2], Joyce Woo[1], Christelle Douillet[3], James G Xenakis[1,4], Rebecca C Fry[4,5], Mirek Styblo[3], Fernando Pardo Manuel de Villena[1,6] [1]Genetics, University of North Carolina-Chapel Hill, [2]Computer Scieince, University of North Carolina-Chapel Hill, [3]Department of Nutrition, University of North Carolina-Chapel Hill, [4]Curriculum in Toxicology and Environmental Medicine, University of North Carolina-Chapel Hill, [5]Department of Environmental Sciences and Engineering, University of North Carolina-Chapel Hill, [6]Lineberger Comprehensive Cancer Center, University of North Carolina-Chapel Hill

In mammals, the arsenic 3 methyl transferase gene (*AS3MT*) encodes the key enzyme responsible for metabolizing arsenic. Humans and mice have a single copy of this gene, but the murine version is significantly more effective in converting inorganic arsenic (iAs) into the less toxic dimethyl- arsenic (DMA). Thus, although laboratory mice have been used for decades as models of arsenic exposure, the ability to translate any finding to humans remains unclear. In 2020, we and our UNC collaborators generated a humanized mouse by replacing the entire mouse *As3mt* gene and its regulatory sequences with the homologous human sequence and demonstrated that these mice metabolize iAs in human-like fashion. To evaluate whether genetic background has any effect on iAs mediated disease phenotypes including metabolic phenotypes, we generated multiple distinct congenic mouse strains carrying the identical humanized locus but otherwise variable background genome wide. We exposed both male and female mice from four congenic strains, B6Crl.129S6-*hAs3mt (*B6), CC003.129S6-h*As3MT* (CC003), CC040.129S6-h*As3mt* and CC057.129S6-h*As3mt* to three doses of (iAs) in deionized drinking water (0.1, 1 or 5ppm) in addition to controls (0 ppm). For each mouse we measured body weight every 2 weeks, body composition (fat and lean mass), fasted

blood glucose (FBG), and blood glucose at 15 minutes (BG15) after glucose challenge, prior to exposure, at the end of the experiment and at midpoint. Mice were fed a semi-purified chow with either low or high fat content. Two examples of strain effects on these phenotypes are: 1) Exposure of female mice to 1 ppm iAs leads to a significant increase in body weight and lower FBG in CC003 but not in B6; and 2) Exposure of male mice to 5 ppm iAs leads to a significantly higher BG15 in CC003 but not in B6. In addition to obvious sex effects, 1 ppm and 5 ppm doses lead to opposite outcomes for weight, FBG, and body composition independently of the strain. This preliminary data using just two congenic strains and two doses show a significant effect of genetic background in metabolic phenotypes after iAs exposure in humanized *As3mt* mice.

1563F **Defining the molecular mechanism of *NARS1*-mediated dominant neurological disease** Sheila Marte[1], Anthony Antonellis[1,2] [1]Human Genetics, University of Michigan, [2]Neurology, University of Michigan

Aminoacyl-tRNA synthetases (ARSs) are essential enzymes that ligate amino acids to cognate tRNAs in the cytoplasm and mitochondria. Although ARSs are essential and ubiquitously expressed, loss-of-function (LOF) missense mutations in six dimeric ARS enzymes have been associated with dominant inherited neuropathy. In addition, heterozygosity for missense and protein-truncating variants in asparaginyl-tRNA synthetase (*NARS1*; the gene encoding the enzyme that ligates asparagine to tRNA molecules in the cytoplasm) have been identified in patients with early-onset neurodevelopmental delay. Thus, ARS genes cause a spectrum of dominant neurological diseases. Interestingly, all ARS enzymes implicated in dominant neurological disease (including NARS1) function as dimers and we previously showed that neuropathy-associated alanyl-tRNA synthetase (*AARS1*) alleles act via a dominant-negative mechanism.

Here, we test pathogenic *NARS1* variants for a dominant-negative effect to determine if this mechanism is common to ARS-associated dominant neurological disease. We performed yeast complementation assays to test R322L and R534* *NARS1* in isolation, which revealed that both alleles exhibit a loss-of-function effect. To test for dominant-negative properties of ARS-mediated neurological alleles, we developed a humanized yeast assay to co-express human mutant *NARS1* with wildtype human *NARS1*. These studies revealed that both mutant alleles repress the ability of the wild-type allele to rescue yeast cell growth, consistent with a dominant-negative effect. To test the neurotoxic effects of these alleles, we developed a *C. elegans* model heterozygous for R534* *NARS1*; we will next characterize this strain and test if overexpression of wild-type *NARS1* rescues any neurological phenotypes. Here, I will present my unpublished data on disease-associated *NARS1* variants, which will provide insight into pathogenic mechanisms.

1564F **Excess accumulation of polyphosphate causes a short replicative lifespan in budding yeast** Chiharu Umeda, Yukio Mukai Graduate School of Bioscience, Nagahama Institute of Bio-Science and Technology

The budding yeast *Saccharomyces cerevisiae* provides numerous insights into cellular aging and lifespan research. The replicative lifespan of the yeast is defined as the number of cells produced by a cell before dying and is unique for the accurate determination of an individual cell's lifespan. Most genetic and environmental factors related to replicative lifespan have been identified, such as calorie restriction, NAD-dependent deacetylase sirtuin and target of rapamycin pathways.

We previously demonstrated that deletion of *PHO80* (cyclin) and *PHO85* (cyclin-dependent protein kinase) genes, which negatively regulate phosphate starvation-responsive genes, drastically shortened the replicative lifespan. The shortened lifespan of the *pho80*Δ mutant was rescued by additional deletion of *PHO4* (transcriptional activator), suggesting that overexpression of the Pho4p target genes causes the shortened lifespan. To identify lifespan-related genes, a transcriptome analysis screened upregulated genes in the *pho80*Δ mutant, and we focused on the *VTC* genes, which encode the vacuolar polyphosphate (polyP) polymerase complex. PolyP, a linear polymer consisting of hundreds of orthophosphate residues for phosphorus and energy storage, mainly accumulates in the vacuole in yeast. The *pho80*Δ mutant was confirmed to highly accumulate intracellular polyP. Deletion of *VTC1*/*VTC2*/*VTC4* restored the lifespan and polyP levels in *pho80*Δ. In the wild type, overexpression of *VTC5* or a combination of the other *VTC*s caused high polyP accumulation and a shortened lifespan. Similar phenotypes were caused by the deletion of polyP phosphatase genes—vacuolar *PPN1* and cytosolic *PPX1*. The polyP-accumulating *pho80*Δ strain exhibited oxidative and heavy-metal stress sensitivities. Thus, we demonstrated that polyP metabolic enzymes participate in yeast replicative lifespan, and high accumulation of polyP leads to a shortened lifespan. Furthermore, the expression of the *Escherichia coli* polyP kinase gene *Ecppk1* shortened yeast lifespan, which was improved by *PPX1* overexpression. Taken together with sequestration of polyP in the vacuole, these data suggest that an increase in cytosolic polyP accelerated the cellular aging process.

1565F **New instrumentation and software for high-resolution, high-throughput yeast fitness profiling to measure genetic interaction globally, discover phenomic modules, and model genetic buffering of disease** Sean Santos, John Rodgers, Jingyu Guo, Ryan Mancinone, John Hartman IV Genetics, Univ Alabama, Birmingham

Phenotypic dependencies involving combinations of functionally variant loci and/or external perturbation are called genetic

interaction and influence disease phenotypes in largely unknown ways. Evolutionarily, genetic interaction contributes population phenotypic variance that is subject to natural selection and underlies differential phenotypic robustness and variable buffering of disease between individuals. Genomic mutant collections are a powerful resource to quantify gene interaction globally for better predicting complex phenotypes across different species and cell types. Cell proliferation is the fundamental fitness phenotype of single-cell eukaryotes like *S. cerevisiae*, and it is resolved with highest precision by growth curves. To promote growth curve analysis for the genomic library of ~ 6000 mutant *S. cerevisiae* strains, we developed a time-lapse imaging instrument to monitor cell proliferation for ~60,000 cultures per experiment. Data, fit ($R^2 > 0.995$) to a logistic growth curve model, yield growth curve parameters to quantify genetic interactions rigorously and precisely. Custom software automates analysis of growth curves from cell array images, measurement of gene interaction, and clustering of gene interaction profiles to compare perturbations phenomically. The approach, quantitative high throughput cell array phenotyping (Q-HTCP), involves serial imaging of dilute cultures spotted onto agar, via a custom robotic line scanner integrated with a commercial robotic incubator and custom program logic control (PLC) for experiment management and data organization. Cell array imaging facilitates visualization of raw data to assess quality and directly examine selected spot cultures in a traditional way. The system capacity is 180 384-culture arrays. Fine resolution of genetic interaction aids identification of protein complexes and molecular pathways, and detection of relatively small or subtle phenotypic effects that may be otherwise elusive in a disease-modeling context. Disease-relevant perturbations explored with Q-HTCP include response to chemotherapeutic agents, quiescence maintenance in stationary phase (chronological survival), and modeling of cystic fibrosis (CFTR) disease mutations in the yeast homolog, Yor1. The presentation shares recent efforts making Q-HTCP more user friendly for bench scientists, including a standardized experimental structure to streamline analysis, assure quality control, and integrate results from independent studies. Examples illustrate use of Q-HTCP and yeast phenomics for modeling genetic complexity of human disease and for collaboration across model systems.

1566F **Evolutionary Rescue of Human Disease Mutations** Brooke Dubyna[1], Ryan Vignogna[1], Ethan Perlstein[2], Gregory Lang[1] [1]Lehigh University, [2]Perlara PBC

Individuals affected with rare diseases often face significant challenges in accessing appropriate medical care due to a lack of research focusing on these conditions. Yeast provides a model system for advancing a basic understanding of human disease associated alleles. Using experimental evolution, we previously identified mutations that arose to compensate for disease phenotypes of human pmm2, an ortholog of yeast sec53. We will extend this approach to other human disease genes, focusing on those involved in congenital disorders of glycosylation, mitochondrial dysfunction, and transcriptional regulation. Our results will map local genetic interaction networks centered around disease-associated alleles. A greater understanding of evolutionary processes and the interactions between these genes and pathways will help in developing targeted therapies for disease management in patients with rare disorders.

1567F **Inhibition of NADPH Oxidase 2 Improves Survival in Zebrafish Infected with Influenza A Virus** Brandy-Lee Soos[1], Alec Ballinger[1], Mykayla Weinstein[2], Julianna Grampone[1], Benjamin King[1] [1]Molecular and Biomedical Sciences, University of Maine, [2]School of Biology and Ecology, University of Maine

Influenza A virus (IAV) is a major health concern since it can cause severe lung infections, especially in older adults and individuals with chronic health conditions. The innate immune system is the host's first defense against pathogens, including IAV. The long-term goal of our research is to understand the molecular mechanisms of the innate immune response to IAV infection and use that information to find new antiviral therapeutic targets. Neutrophils have essential roles in innate immunity to bacterial and fungal infections, but their roles in antiviral responses are understudied. Neutrophils generate an inflammatory response following infection, but that response must be carefully regulated. A sufficient level of inflammation is needed to clear infection, but too much inflammation results in a damaging hyperinflammatory response. The zebrafish (*Danio rerio*) is a powerful vertebrate model system that is used to study IAV infection. Zebrafish larvae are used to study innate immune responses as the adaptive immune system is not active until approximately one month of age. Using zebrafish larvae, we study the roles neutrophils have in controlling IAV infection and how over-activation of neutrophils during IAV infection trigger a damaging hyperinflammatory response. During the inflammatory response, reactive oxygen species (ROS) are produced and released by NADPH oxidase 2 (NOX2) in neutrophils through the respiratory burst response. We hypothesize that reducing the respiratory burst response will limit tissue damage and improve survival. Using RNA sequencing, we found that the oxidative stress response pathway was upregulated in IAV-infected larvae compared to uninfected controls at six hours post infection. To test our hypothesis, we compared survival and respiratory burst response in IAV-infected larvae treated with and without a NOX2 inhibitor, GSK205739, to controls. The NOX2 inhibitor improved survival with IAV infection. Respiratory burst assays that measure the capacity of larvae to generate ROS showed an improved response in IAV-infected larvae with NOX2 inhibitor treatment compared to controls. These and other studies will help us identify how inflammation is regulated during IAV infection so that therapeutic measures that preserve the antiviral response, yet contain the associated inflammation, can be developed.

1568F **Zebrafish as a Translational Model of Chronic Early Life Stress: Insights Along the Oral-Gut-Brain Axis** Christina Graves[1], Erik Norloff[1], Darius Thompson[1], Cassandra Sang[1], Oksana Kosyk[2,3], Anthony Zannas[4] [1]Biomedical Sciences, UNC-Chapel Hill, [2]Genetics, UNC- Chapel Hill, [3]Psychiatry, UNC- Chapel Hill, [4]Psychiatry and Genetics, UNC- Chapel Hill

Chronic early life stress (ELS) is known to alter the development and functioning of the nervous and immune systems and has been associated with a myriad of negative health outcomes along the Oral-Gut-Brain axis. In addition to it's relatively well-described effects on the developing brain, ELS is known to be an important environmental modifier of gut inflammation in humans and has also been associated with the earlier emergence of pre-permanent teeth suggesting that the teeth can serve as a biometric indicator of stress-induced premature aging. Though physiological stress responses are appreciated to have major tissue targets throughout the body, the mechanisms linking ELS to tissue dysfunction later in life is incompletely understood. Here, we employed a novel, prolonged chronic stress paradigm recently developed by our lab (Graves et al. 2023) in which random, unpredictable environmental stressors are initiated at the onset of exogenous feeding and continued throughout larval development mimicking early childhood stress. Behavioral analysis was performed by videographic recording and analysis of free-swimming behavior in a novel tank test and showed that ELS-exposed zebrafish demonstrated a significant increase in anxiety-like behaviors as well as a significant – yet transient – reduction in body size. We found that ELS reduces enteric neuronal density, delays intestinal transit, and results in the differential expression of neuroimmune genes in a tissue- and time-dependent manner suggesting that ELS impacts the enteric nervous system in similar-yet-distinct ways to that of the developing brain. We have also recently established an experimental pipeline to develop zebrafish as a model organism to advance our understanding of the fundamental mechanisms underlying the impacts of prolonged ELS on the developing dentition. By leveraging high-resolution X-ray microtomography (micro-CT) and whole mount tissue clearing immunofluorescent confocal imaging of cleared whole-mount zebrafish (3-DISCO), our preliminary data suggest that ELS disrupts both the timing and morphology of newly emerged pharyngeal teeth. Altogether, our findings provide insights linking ELS with gut dysfunction and disrupted dental development, highlighting the zebrafish model system as a valuable tool in uncovering how "the body keeps the score" along the Oral-Gut-Brain axis.

1569F **Leveraging gametogenesis-specific rejuvenation pathways to counteract cellular aging** Tina L Sing[1], Katie Conlon[1], Stephanie H Lu[1], Nicole Madrazo[1], Tianhao Yin[1], Kaitlin Morse[1], Juliet Barker[1], Ina Hollerer[1], Gloria Brar[1], Peter H Sudmant[2], Elçin Ünal[1] [1]Molecular and Cell Biology, University of California, Berkeley, [2]Integrative Biology, University of California, Berkeley

Aging manifests as an accumulation of cellular defects that eventually cause functional decline, disease, and organismal death. Significant research into the causes of cellular aging has revealed a growing list of age-associated factors that are conserved from yeast to humans. Interestingly, gametogenesis contains inherent rejuvenation pathways that prevent age-associated damage from being passed onto progeny, which leads to lifespan resetting. Thus, there is strong incentive to understand how gametogenesis-specific rejuvenation genes can eliminate aging biomarkers and determine which of these pathways can be leveraged to counteract cellular aging in somatic cells. Gametogenesis is a highly regulated developmental program whereby diploid progenitor cells undergo cell division (meiosis) and differentiation to produce haploid gametes; however, the complete complement of gametogenesis genes involved in cellular rejuvenation remains largely unknown. Our lab and others are working to identify and understand how gametogenesis-specific mechanisms remove aging biomarkers to reset lifespan in *Saccharomyces cerevisiae*. Excitingly, overexpression of the meiotic transcription factor, Ndt80, is sufficient to extend lifespan suggesting that at least a subset of gametogenesis-specific rejuvenation pathways can be repurposed for use outside of their natural context (reviewed in Sing et al., 2022a).

We have constructed 5 inducible cDNA libraries (Sing et al., 2022b) and developed a screening pipeline to measure the effect of gametogenesis genes on competitive fitness in both young and old yeast cells. This led to the identification of 80 rejuvenation gene candidates, including genes with roles in different organelles (e.g. mitochondria, endoplasmic reticulum, Golgi apparatus, and vacuole) and genes with diverse biological functions (e.g. genome maintenance, RNA processing, cell cycle regulation, metabolism, and stress response). We believe that the diversity in this list is exciting because it may represent multiple pathways that converge to rejuvenate aging cells. We also found several rejuvenation candidates that are potential targets of Ndt80, which may facilitate nucleolar rejuvenation and lifespan extension that has been previously reported. Currently, we are using a microfluidic pedigree system to perform single-cell lifespan measurements in strains overexpressing each of the rejuvenation candidates identified in our screen. Future research will be focused on: (i) understanding how these genes promote cellular rejuvenation; (ii) determining if there is crosstalk between these rejuvenation genes; and (iii) optimizing gene dosing and synergy to promote longevity.

1570S **Lifespan and mitochondrial effects of 6PPD in *Caenorhabditis elegans*** Steven Beck[1], Laxmi Rathor[2], Taylor McElroy[1], Stephanie Wohlgemuth[1], Myon Hee Lee[3], Moon Jung Hyun[4], Sung Min Han[1] [1]Physiology and Aging, University of Florida, [2]Physiological and Aging, University of Florida, [3]Eastern Carolina University, [4]Korea Institute of Toxicology

The synthetic antioxidant N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) is commonly utilized in rubber-based products like tires, and its release into the environment during tire wear poses a threat to wild salmon populations. However, the toxicity of 6PPD in other species and the underlying mechanisms still need to be better understood. In this study, we investigated the potential toxicity and targets of 6PPD using the in vivo model system C. elegans. Exposure to 0.5 mM 6PPD in C. elegans resulted in adverse effects, including delayed development, decreased body growth, and reduced reproduction. Additionally, 6PPD exposure negatively impacted healthspan parameters, such as body motility and stress tolerance, leading to a shortened lifespan. 6PPD-exposed C. elegans also exhibited disrupted mitochondrial function, characterized by reduced mitochondrial membrane potential, lower oxygen consumption, diminished ATP levels, and decreased reactive oxygen species (ROS). Notably, 6PPD exposure influenced the activity of SKN-1/Nrf2, a key transcription factor involved in stress response and longevity. Suppression of SKN-1 attenuated reductions in lifespan and tolerance against paraquat, suggesting the involvement of SKN-1/Nrf2 in mediating the toxic effects of 6PPD. Together, these findings provide insights into the potentially toxic effects of 6PPD on development, health, and aging processes, identifying mitochondria as a potential target organelle affected by 6PPD exposure. Understanding the mechanisms behind the toxic effects of 6PPD is crucial for developing strategies to mitigate its environmental impact and protect vulnerable species.

1571S **Roles of purine biosynthesis represented in disease models of perturbed *de novo* and salvage synthesis** Maia Pappadakis, Mia Peifer, Adam Fenton, Wendy Hanna-Rose Pennsylvania State University

Purines, essential molecules to many biological processes, are produced by *de novo* synthesis and salvage synthesis pathways. Both of these pathways are highly conserved across organisms, evidence that each is important in maintaining purine homeostasis. Mutations in *de novo* and salvage synthesis enzymes are associated with Mendelian diseases in humans. Two examples of these diseases are adenylosuccinate lyase deficiency (ASLD), the result of a deficiency of the *de novo* enzyme adenylosuccinate lyase (ADSL), and Lesch-Nyhan disease, the result of a deficiency of the salvage enzyme hypoxanthine-guanine ribosyl transferase (HGPRT). We use the *Caenorhabditis elegans* model to study these diseases associated with purine synthesis, where all purine pathway enzymes have orthologs. In both human disorders and *C. elegans* models, phenotypes are quite distinct both between and within loss of function in *de novo* and salvage pathway enzymes.

In humans, ASLD causes phenotypes such as seizures and reduced psychomotor function where Lesch-Nyhan causes phenotypes such as self-injurious behaviors and gouty arthritis. In *C. elegans* models, *adsl-1* deficiency causes infertility and reduced motility where *hprt-1* deficiency causes no observable fertility or psychomotor phenotypes. These distinct phenotypes suggest specific biological roles are interrupted by the loss of function of these *de novo* or salvage pathway enzymes, and imply that intermediary purine metabolites may also play important roles. One curious observation in both human and *C. elegans* cases of ADSL/*adsl-1* loss of function is that while we observe significant phenotypes, there is no change in steady-state purine levels. Additionally, there is an increase in *hprt-1* expression in *adsl-1* loss of function, leading us to hypothesize that compensatory mechanisms between *de novo* and salvage pathways play a role in disease mechanisms. I aim to study the interactions between *de novo* and salvage synthesis that maintain purine levels by measuring the regulatory response of the salvage pathway in *de novo* loss of function and vice versa. Yet another interesting preliminary observation is a reduced fat content in the intestine of *hprt-1* loss of function *C. elegans*. I will report on experiments to examine the function of salvage in the etiology of *adsl-1* loss of function phenotypes and the phenotypic and metabolic analysis of *hprt-1* loss of function.

1572S **How do the Orsay virus and its variants affect the Intracellular Pathogen Response in *C. elegans?*** Abigail Rose Reese[1], Nicolas Martinez[2], Sehrish Khan[2], India Cannon[2], Jessica N Sowa[2] [1]Biology, West Chester University of Pennsylvania, [2]West Chester University of Pennsylvania

In 2011, the Orsay virus was discovered as the first virus known to naturally infect *C. elegans*. This is a ssRNA, non-enveloped, +sense virus that contains 4 proteins including capsid, alpha-delta, delta, and RDRP. Infection of *C. elegans* by this virus activates an innate immune response called the Intracellular Pathogen Response (IPR), which is a set of 80 genes that are transcriptionally upregulated. This response can also be triggered by heat, stress, other intracellular pathogens, or Bortezomib. Bortezomib is a chemotherapy drug that activates the *C. elegans* IPR when exposed, and preliminary data from our lab indicates that when worms are exposed to Bortezomib and the Orsay virus, the IPR levels decrease relative to Bortezomib exposure alone. This suggests that the IPR is lessened when the worms are infected with the Orsay virus.

Our current study seeks to understand how the Orsay virus affects the IPR in *C. elegans.* Evidence suggests that the Orsay virus dampens the IPR, so we are interested in determining which of the 4 Orsay proteins is responsible for this. Previous evidence has shown that the RDPR upregulates the IPR, not represses it, so we created transgenic strains that can be used to test the capsid, alpha-delta, and delta proteins individually. We are also investigating how variation in the virus affects the IPR by comparing infection rates in different Orsay variants. Our data indicates that divergent Orsay variants V2 and V13 show lower infectivity rates, and we are also assessing the effect of these variants on the IPR. Overall, our results add to our knowledge

of how this virus interacts with its' host, helping to guide and expand the use of *C. elegans* as a model organism for host-virus interactions.

1573S **Variation in Orsay virus affects progeny numbers of *C. elegans*** Solon Aguila, Sehrish Khan, Jessica Sowa West Chester University

C. elegans has been used as a model organism to study the innate immune system. To study their innate immune responses, they need to be infected and the only virus known to naturally infect *C. elegans* is the Orsay virus. Since the original discovery of the Orsay virus there have been several different variants of Orsay discovered in Europe. Two of those variants we discovered from samples collected in the Netherlands, and we named these variants V2 and V13. To compare V2 and V13 variants to the original Orsay isolate JU1580 we wanted to compare how they can affect host fitness. We have found that Orsay JU1580 decreases the number of progenies from infected worms. Previous experiments done by others looking at progeny effects did not find a difference in the overall number of progenies, but they found a delay in progeny production. The decrease in offspring we found in Orsay JU1580 infected *C. elegans* could be from the delay in progeny production. A previous experiment our lab has performed comparing V2 and Orsay JU1580 progeny numbers found that V2 did not affect the number of progenies produced. I am now comparing the different effects of Orsay JU1580 and its variant V13 have on the fertility of *C. elegans*. The organisms were time synchronized through bleaching and plated on three infection plates, one control, one Orsay JU1580, and one V13. Three adults were moved to normal NGM plates and left for two days to procreate in triplicate. The adult worms were removed and then the progenies were left to grow for two days. The progenies were then counted and compared using one-way ANOVA. Our results show that the average number of progenies produced by Orsay JU1580 infected *C. elegans*, 392 ±53 progeny, is significantly smaller than the average number of Orsay V13 infected, 188 ±106 progeny, and control *C. elegans*, 402 ±64 progeny. There was no statistically significant difference between the progeny count of V13 and the control group. The data was analyzed using a one-way ANOVA ($p=0.0013$, $n=3$). Based on these results, we concluded that V13 does not affect *C. elegans* fertility whereas Orsay JU1580 does.

1574S **Predictive modeling to define the locus heterogeneity of tRNA synthetase-related peripheral neuropathy** Allison Cale[1], Anthony Antonellis[2] [1]Human Genetics, University of Michigan, [2]University of Michigan

Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed, essential enzymes that ligate amino acids to tRNAs. Variants in six genes encoding an ARS cause dominant axonal peripheral neuropathy, which presents the question: how do variants in ARSs, which are essential in all tissues, lead to phenotypes restricted to the peripheral nervous system? While defects in protein translation and activation of the integrated stress response have been implicated downstream of neuropathy-associated ARS variants, a unifying pathological mechanism that explains the locus heterogeneity has not been identified. All six neuropathy-associated ARSs function as dimers, which is consistent with a dominant-negative effect.

Seryl-tRNA synthetase (*SARS1*) is a dimeric ARS implicated in dominant peripheral neuropathy. We tested the pathogenic S396F *SARS1* allele for loss-of-function and dominant-toxicity in humanized yeast assays. We found that when the yeast ortholog *SES1* is repressed, S396F *SARS1* does not complement yeast growth, indicating it is a loss-of-function mutation. Furthermore, when co-expressed with wild-type *SARS1*, S396F *SARS1* reduces yeast growth, indicating that it exerts a dominant toxic effect on wild-type SARS1.

If a dominant-negative effect is the primary disease mechanism of ARS-mediated dominant peripheral neuropathy, then certain variants in any dimeric ARS could exert a dominant-negative effect and lead to dominant neuropathy. To test this, we engineered missense mutations (based on conservation and localization to functional domains) in threonyl-tRNA synthetase (*TARS1*), a dimeric ARS not yet implicated in neuropathy. We have tested seven variants to date: (**1**) five are loss-of-function alleles; and (**2**) two repress wild-type *TARS1*, consistent with a dominant-negative effect.

Based on these data, we generated two heterozygous *C. elegans* models to test if dominantly toxic *SARS1* and *TARS1* alleles cause dominant neurologic and motor behavior phenotypes in a multicellular organism. By using a two-pronged approach examining a *bona fide* pathogenic allele in *SARS1*, combined with a predictive modeling strategy of identifying dominantly toxic *TARS1* alleles, we aim to elucidate the mechanism by which numerous alleles across six ARS genes converge on a dominant peripheral neuropathy phenotype. Here, I will present all of my unpublished data on these studies.

1575S **TCER promotes embryonic health upon maternal infection** Laura Bahr[1], Danny Bui[2,2], Francis RG Amrit[3], Andre Viera[4], Carissa Olsen[4], Arjumand Ghazi[1] [1]Rangos Research Center, Departments of Pediatrics, Developmental Biology and Cell Biology & Physiology, University of Pittsburgh School of Medicine, [2]University of Pittsburgh, [3]Rangos Research Center, Departments of Pediatrics, Developmental Biology and Cell Biology & Physiology, University of Pittsburgh, [4]Departments of Chemistry and Biochemistry, Worcester Polytechnic Institute

Fertility and immunity are both energy intensive metabolic programs. Energy allocation by an organism to one process is a trade-off at the cost of the other. Lipids are an energy-rich resource whose mobilization is integral to both reproductive success and pathogen defense. We are investigating the role of lipid-metabolic pathways in the immunity-fertility dialogue through infection of the nematode *Caenorhabditis elegans*, a well-established molecular-genetic model organism, by the human opportunistic pathogen *Pseudomonas aeruginosa* (PA14).

Previously, we demonstrated that PA14 infection dramatically reduces fertility and that TCER-1, worm homolog of a human transcription elongation and splicing factor TCERG1, protects host reproductive fitness in the presence of pathogen.

Our recent data demonstrate that PA14 infection causes rapid depletion of stored lipids in somatic tissues, but that lipid stores in developing embryos are protected from depletion by TCER-1. Conversely, TCER-1 inhibits lipid deposition in the maternal hypodermis; which significantly does not mobilize lipids to the germline. Thus, TCER-1 diverts maternal energetic resources away from somatic storage toward reproduction. Furthermore, we show that TCER-1 promotes higher levels of polyunsaturated and monomethyl branched chain fatty acids in embryonic tissue upon PA14 infection. These fatty acids are required for formation of the innermost eggshell layer, the lipid permeability barrier. Accordingly, upon PA14 infection we observe a partially penetrant failure to form the lipid permeability barrier in *tcer-1* embryos, which is lethal. Together, this data demonstrates that TCER-1 promotes lipid allocation to embryos, and ensures their viability upon maternal infection with PA14.

1576S **Control of mitochondrial DNA purifying selection by regulators of aging and programmed cell death** Joel H Rothman, Sagen Flowers, Samantha Fiallo, Abhayjit S Saini, Rushali Kothari, Yamila Torres Cleuren, Melissa Alcorn, Chee Kiang Ewe, Geneva Alok, Pradeep M Joshi MCD Biology, University of California

Animal cells harbor many copies of the mitochondrial genome (mtDNA) and multiple mtDNA genome variants can be stably maintained in each cell. This heteroplasmy allows deleted mtDNA mutants to accumulate cryptically through replicative advantage. mtDNAs undergo high rates of mutation and defective mtDNAs, which accumulate during aging, are associated with a range of age-related diseases. Mechanisms to remove defective mtDNAs through mitochondrial “purifying selection” (MPS), notably mitophagy, function to ensure fidelity of mtDNA, particularly during development of the maternal germline, the source of the mtDNA that is inherited by the next generation. We found that pro-apoptotic regulators, including the CED-3 and CSP-1 caspases, the BH3-only protein CED-13, and engulfment factors, are required in *C. elegans* to attenuate germline abundance of an mtDNA deletion (*uaDf5*), which is otherwise stably maintained in heteroplasmy with wildtype mtDNA. In contrast, removal of pro-apoptotic CED-4/Apaf1 does not increase accumulation of the deleted mtDNA, suggesting a non-canonical role for CED-13 and the caspases. While we found that *uaDf5* increases the number of cell corpses observed in adult germlines, elimination of apoptotic cell death does not alter the burden of defective mtDNA, suggesting that a caspase-dependent, cell death-independent mechanism is required for MPS. We observed that the load of germline *uaDf5* increases with maternal age, and that this higher burden is passed onto the offspring, with only partial intergenerational removal of the defective mtDNA; thus, older mothers produce offspring with higher levels of mutant mtDNA. In mutants with the highest *uaDf5* burden, the intergenerational MPS is enhanced in older mothers, suggesting an age-induced mechanism of mtDNA quality control. Older mothers bearing the defective mtDNA also show more rapid decline in germline morphology, consistent with more rapid aging of this tissue compared to animals that carry only intact mtDNA. Further, we found that age-dependent accumulation of *uaDf5* is decreased in long-lived, and increased in short-lived, mutants. Thus, regulators of both PCD and the aging program are required for germline mtDNA quality control and its intergenerational transmission. Finally, we obtained evidence that different mtDNA mutants are eliminated by distinct mechanisms, suggesting that MPS requires multiple pathways to ensure that a variety of defects of the mitochondrial genome are removed.

1577S **Modulating material properties of cargo protein to probe exopher biology** Edward Chuang[1], Mayur Barai[2], Benjamin Schuster[2], Monica Driscoll[1] [1]Molecular Biology and Biochemistry, Rutgers University, [2]Chemical and Biochemical Engineering, Rutgers University

Propagation of pathological protein species is a hallmark of neurodegenerative disease progression. The mechanism of cell-to-cell transfer and the nature of the pathological protein species that spread in disease are not well understood. While protein aggregates are observed in postmortem tissue from neurodegenerative disease patients, many of the same proteins also form smaller oligomers or liquid and hydrogel condensates. In this work, we hypothesize that exophers act as a potential mechanism for proteins to spread from cell to cell. Exophers are large extracellular vesicles that were first observed in *C. elegans* and have since been observed in other mammalian systems. An exopher is generated when a cell extrudes up to half of its volume into a large membrane-bound vesicle that contains protein aggregates and damaged organelles. Notably, exophers preferentially recruit aggregates composed of mCherry and Htt-polyQ128, and preferentially exclude soluble GFP. Selection and recruitment of exopher cargo may be sequence-specific or a consequence of the aggregated state of the protein. Furthermore, it is unknown if exophers preferentially recruit liquid or hydrogel protein condensates that have intermediate material properties

between soluble and aggregated protein. To address this question, we express PGL-3, a native *C. elegans* germline protein, as a model substrate in our established touch neuron model of exophergenesis. We show that PGL-3 condensates are extruded in exophers but may also remain in the neuronal soma. We mutate the intrinsically disordered region of PGL-3 and characterize the altered phase behavior *in vitro* and *in vivo*, and we determine the effects of these mutations on exophergenesis and PGL-3 cargo recruitment to exophers. These findings further our understanding of exopher cargo criteria and condensate biology in the neurons of living animals.

1578S **RareResolve - Gene-humanized Animal Models for Discovery of Variant Pathology in Variants of Uncertain Significance (VUS).** Christopher Hopkins, Trisha Brock, Kathryn McCormick, Benjamin Jussila, Sarah Cheesman InVivo Biosystems

Whole genome sequencing is rapidly being adopted in the clinic and the rate of data acquisition into the ClinVar database continues to grow exponentially. Especially impacted are the Variants of Uncertain Significance (VUS) which are growing exponentially while the remaining categories of Pathogenic, Likely Pathogenic, Benign, Likely Benign show a small (linear) yearly increase. The result is unactionable VUS are forecast to take over the database by the end of the decade. The clinician may see a variant in their patient, but after cross reference to ClinVar, no clear guidance will be obtained. ACMG-AMP guidelines require functional studies to be performed as one of the major ways to enable reclassification of a VUS. We have developed a gene humanization methodology in C. elegans and Zebrafish as a way to quickly and affordably generate functional data that resolves VUS. In a C. elegans demonstration (Hopkins et al., 2023 Genetics in Medicine Open), over 80 variants were installed into a STXBP1-humanized locus and a diagnostic curve (AUC: 0.940) was generated, which enabled functional assessment of 5 of 23 VUS as pathogenic (21% yield). These results are in concordance with bioinformatics methods for predicting pathogenicity in VUS. Using AlphaMissense (AM) (Cheng et al., 2023, Science), we found our 5 pathogenic assessments of VUS were fully concordant. A second method using Gibbs Free Energy (GFE) calculations observed 4 of the 5 variants as pathogenic (80% concordance). However, the additional concordant calls for pathogenicity between AM and GFE suggests 3 variants were missed in the C. elegans model. Since our C. elegans method failed to achieve functional data to support for all 8 predicted variants, we have started studies to use transient expression studies in zebrafish (Crispant F0 knockouts with human mRNA rescue) as an alternative method to generate functional data. Better concordance is expected because functional testing in zebrafish can be performed closer to the physiologically-relevant temperature of humans (37 degree C). In conclusion, the combined use of these two animal models creates the RareResolve platform, which provides an on-demand diagnostic system to resolve pathogenicity in VUS seen in genomic sequencing of the patient.

1579S **Multifactorial *C. elegans* depression model** Dianelena Eugenio-Perez[1], Francisco D Dirzo-Diaz[2], José Pedraza-Chaverri[3] [1]Biology, Faculty of Chemistry UNAM, [2]Faculty of Chemistry, UNAM, [3]Biology, Faculty of Chemistry, UNAM

Current animal models of psychiatric diseases do not adequately model these pathologies mainly because they do not share the complex etiological origin of psychiatric illnesses. In this regard, a *C. elegans* model could be an alternative since combinations of genetic and environmental factors can be easily modelled. Furthermore, because of its short life cycle (3 days), factors affecting different developmental stages can be included in experimental models.

This presentation will describe a *C. elegans* depression model that includes both a genetic and an environmental factor, the latter being applied in the first larval stage (L1). The genetic factor is the usage of a knockout *npr-19* strain. Notably, the human homologue gene *CNR-1* has been reported to be altered in depressed patients. Concerning the environmental factor, we repeatedly induce the flight response in worms by periodically tapping their Petri dish with a microcontroller-driven solenoid. This repeated induction of stress response models chronic stress which is a relevant predictor of depression. Importantly, this factor is applied at early life because the link of early life stress and depression is well documented.

In this poster, we will present detailed results on the impact of the genetic, environmental and the combination of both factors in motivated behaviour of *C. elegans* adults, specifically in motivated food seeking, where worms are food-deprived and spontaneous movement for food seeking is assessed. Moreover, we describe the impact of each factor and their combination on redox status, which is known to be a relevant pathophysiological contributor of depression.

1580S **The broccoli derivative sulforaphane increases lifespan across diverse genetic backgrounds of *C. elegans* and resets the transcriptional aging clock toward a "youthful" state** Patrick C Phillips[1], Christine Sedore[2], Anna Coleman-Hulbert[2], Erik Johnson[2], Erik Segerdel[2], Brian Onken[3], David Hall[4], Theresa FitzGibbon[4], Yuhua Song[3], Hadley Osman[4], Madhuri Achanta[3], Elena Battistoni[4], Stephen Banse[2], Gordon Lithgow[4], Monica Driscoll[3] [1]Institute of Ecology and Evolution, University of Oregon, [2]University of Oregon, [3]Rutgers University, [4]Buck Institute for Research on Aging

Sulforaphane (SFN), an organosulfur isothiocyanate derived from cruciferous vegetables, has been shown to inhibit inflammation, oxidative stress, and cancer cell growth; it has also been shown to increase the lifespan of the *C.*

elegans nematode strain N2. Here, we tested the lifespan effects of SFN on an expanded set of three *Caenorhabditis* species, following the standard experimental procedures of the *Caenorhabditis* Intervention Testing Program (CITP). The CITP, a multi-institute research consortium, aims to identify chemical interventions that can robustly and reproducibly promote health and lifespan, testing compounds across a genetically diverse panel of *Caenorhabditis* strains and species. Our results indicate that SFN increases lifespan in a strain- and species-specific manner: *C. elegans* strains N2, JU775, and MY16 all exhibited a significant increase in median lifespan when treated with SFN (59%, 65%, and 47%, respectively), whereas SFN treatment either decreased or did not affect lifespan in six strains of the two *C. briggsae* and *C. tropicalis* species. To investigate the functional targets of SFN, we employed bulk RNA-Seq at a variety of ages to create a "transcriptional aging clock" using control individuals and then tested how global patterns of gene expression are altered under SFN treatment. Multi-dimensional scaling analysis of the transcriptome revealed transcriptional age under SFN treatment was similar to control individuals approximately four days younger, representing a nearly 20% shift relative to overall lifespan. These results support the idea that robust longevity-extending interventions can act via global effects across the organism, as revealed at a functional level via changes in gene expression.

1581S **Mitochondrial fusion promoter M1 molecule is sufficient to enhance mitochondrial function and organismal lifespan** Julia Bailey, Laxmi Rathor, Taylor McElroy, Sung Min Han University of Florida

Mitochondria play a crucial role in regulating organismal aging and health. Existing studies suggest a correlation between age-related decline in mitochondrial function and aging. Improving mitochondrial activity and homeostasis has been proposed as a potential strategy to mitigate the effects of aging. However, there is currently insufficient knowledge about the effectiveness of pharmacological manipulation of mitochondrial homeostasis in enhancing the lifespan and healthspan of a live animal.

Our study focuses on M1, a small molecule recognized for its ability to promote mitochondrial fusion and transport. To investigate the impact of M1 on mitochondrial homeostasis and organismal aging, we employed the nematode C. elegans as an in vivo model. Our findings indicate that M1 enhances mitochondrial membrane potential and reduces reactive oxygen species levels. Notably, M1 treatment results in a dose-dependent lifespan extension in worms, while the accumulation of lipofuscin, an aging pigment, remains unchanged.

These results provide promising insights into M1 as a potential modulator of animal lifespan. Our ongoing research aims to explore whether M1 requires mitochondria to influence lifespan and to assess its broader impact on various healthspan parameters, including mobility and stress tolerance.

1582S **ImR tumor induces kidney damage in adult *Drosophila* hosts Fei Cong, Hong-Cun Bao, Wu-Min Deng. Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, New Orleans, LA** Fei Cong, Hongcun Bao Tulane University

Kidney damage is one of the paraneoplasia symptoms in cancer patients. Compared to those without CKD (chronic kidney damage), cancer patients with CKD may have an increased risk of death. However, the relationship between CDK and cancer is unclear. Here, using the transplanted ImR tumor model, we explored the mechanism underlying kidney damage in adult *Drosophila* hosts.

Previously, we demonstrated that overexpression of the Notch receptor's functional domain in the transition zone of larval salivary imaginal ring (ImR) can induce tumorigenesis through polyploid mitosis and depolyploidization. To sustain the growth of larval ImR tumors continuously, our lab has developed a new system for transplanting small tumor pieces into the abdomen of adult flies and passaging the tumor tissue across generations.

In tumor-host flies, we observed an increase in the size of pericardial nephrocytes and a decrease in their numbers. Pericardia nephrocytes, located in the posterior region of adult flies, is one of the two renal organisms in *Drosophila* which performs same function of podocyte in human nephron. Both podocytes and nephrocytes have structures known as slit diaphragms that filter hemolymph through endocytosis. Pyd, one of the component proteins of slit diaphragm forming a fingerprint pattern in *Drosophila*, is involved in endocytosis with other endosome proteins like Rab5. Data show that the Pyd expression pattern is disrupted, and Rab-family genes expression level are altered in nephrocytes of tumor-host flies. To determine the cause of nephrocyte destruction and loss, we used various methods to demonstrate that the function of host nephrocyte is more active than that of controls. Additionally, due to impaired degradation ability, toxin factors accumulate in nephrocytes, ultimately leading to inflammation and apoptosis.

To further emphasize the importance of nephrocyte function in tumor hosts, we use *klf15* mutant flies that lack nephrocytes in the adult stage as tumor hosts. Compared to control hosts, the lifespan of *klf15* mutant hosts was significantly reduced. Taken together, our results indicate that nephrocytes play a crucial role in prolonging host survival, and we provide new insights into

the mechanism of kidney damage in cancer.

1583S **A *Drosophila* Genetic Screening Platform to Identify Conserved Factors that Influence Biological Outcomes Following Traumatic Brain Injury Exposure** Jesse Rojas[1], Eddie Cho[1], Alec Candib[1], Robert Squire[1], Elizabeth Chai[1], Marta Lipinski[2], Kim Finley[1] [1]San Diego State University, [2]University of Maryland School of Medicine

Following traumatic brain injury (**TBI**) exposure a wide range of factors including genetic have the potential to influence the biological outcomes. Millions of people worldwide sustain a wide range of head trauma, resulting in diverse outcomes. The potential for genetic factors to influence TBI outcomes requires further studies. Here we use a high throughput *Drosophila* screening platform to elucidate key genes and pathways that may influence TBI outcomes. This included the use our BeadRuptor trauma technology and validated TBI phenotypes, along with the *Drosophila* Gal4-UAS system to modulate select genes in adult fly neural and glia tissues. RNAi knockdown (**KD**) technique was used to target autophagy (***ref(2)P***), lipidomic (***lipase3***), glycogenic (***shaggy***), and inflammatory (***crq***) pathway components. Adult cohorts were exposed to severe (**sTBI, 1x**) or mild repetitive trauma (**mTBI, 10x**) conditions to assess individual genetic changes to TBI outcomes. Biological assessments included modified longevity profiles, behavioral changes (negative geotaxis response, **NGR**), alterations to protein turnover (**autophagy**), and inflammatory profiles. Mortality indexes (**MI^{24hr}**) and aging studies show genotypic dependent changes, reflecting both sensitivity and resistance to following trauma exposure when compared to wildtype (**WT**) controls. Alterations to climbing behaviors (**NGR**) also showed similar genotype dependent responses following mTBI (**10x**). Neural Western analysis established basal and fasting induced alteration to autophagic responses. Further, each RNAi KD showed genotype dependent impact on NF-κB signaling targets (**AMP**). This included basal inflammatory profiles as well as following primary (4hr post mTBI) and secondary (24hr post mTBI) injury responses. In summary, there was a genotypic specific concordance between longevity and locomotor profiles following trauma, along with unique proteolytic, and neuroinflammatory responses highlighting potential key molecular mechanisms. This work demonstrates the versatility of *Drosophila* as a genetic screening platform to examine neural responses and outcomes following trauma. Genes examined here have human homologues and known genetic variants, highlighting potential targets for further investigation of trauma patients.

1584S **Dominant *OGDH* variants cause peripheral neuropathy with ataxia and optical atrophy** Wan Hee Yoon[1], Liedewei Van de Vondel[2,3], Juan Felipe Ramirez[1], Jonathan De Winter[2,3,4], Satoshi Matsuzaki[1], Abigail Sandoval[1], Sukyeong Lee[5], Rita Horvath[6], Kenneth M. Humphries[1], Jan de Bleecker[7], Stephan Züchner[8], Jonathan Baets[2,3,4] [1]Aging & Metabolism Research Program, Oklahoma Medical Research Foundation, [2]Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp, [3]Laboratory of Neuromuscular Pathology, Institute Born-Bunge, University of Antwerp, [4]Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, [5]Department of Biochemistry & Molecular Biology, Baylor College of Medicine, [6]Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, [7]Department of Neurology, University Hospital Ghent, [8]Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine

2-oxyglutrate dehydrogenase (OGDH) is an E1 component of α-ketoglutarate dehydrogenase complex (α-KGDH) that plays a pivotal role in the Krebs cycle metabolism. Biallelic variants in *OGDH* have been reported to cause OGDH deficiency (OGDHD; OMIM: # 203740), an early-onset neurodevelopmental and mitochondrial disorder. However, whether monoallelic *OGDH* variants could lead to dominant effects in humans had not been known. In this study, we identified *de novo OGDH* c. 1909C>T (p.Arg637Trp) and heterozygousc.162T>G (p.Ser54Arg) variants by whole-exome sequencing (WES) in individuals exhibiting late-onset neurological phenotypes, including peripheral neuropathy, cerebellar ataxia and bilateral optic atrophy. Blood analysis for the individual with the p.Arg637Trp variant revealed ketogenosis. In patient lymphoblasts, OGDH protein levels did not appear altered, both in whole-cell lysate and in the mitochondrial fraction, compared to familial controls. In silico protein structure predictions using AlphaFold and homology modeling suggest that p.Arg637Trp mutation might influence protein structure and solubility. To determine whether the monoallelic *OGDH* variants act as dominant-negative mutations, we generated *Drosophila* models harboring *UAS-dOgdh (p.Arg639Trp)* and *UAS-dOgdh (p.Thr58Arg)* mutations, homologous to the human variants. We found that ubiquitous expression of *dOgdh (p.Arg639Trp)* or *dOgdh (p.Thr58Arg)* did not result in developmental lethality. While the mutant OGDH expression by *dOgdh-T2A-Gal4* driver did not lead to defects in development or locomotion in young adults (3-day-old), it did lead to locomotion defects in aged flies (14-day-old). These findings indicate that both variants are hypomorphic alleles and act as document-negative mutations, consistent with the observed clinical manifestations in individuals carrying these monoallelic *OGDH* variants. Our data suggest that monoallelic *OGDH* variants disrupt α-KGDH function, leading to a late-onset neurological disease in humans.

1585S **Utilizing Drosophila to Understand Second Heart Field Development** Brenna Blotz, Richard M Cripps Biology, San Diego State University

Many congenital heart defects result from the abnormal development of a progenitor cell type known as the second heart field (SHF). Heart defects arising from SHF malformation can be a symptom of 22q11.2 Deletion Syndrome (22q), which is a human disease resulting from a microdeletion on chromosome 22. The origins and importance of the second heart field continue to be firmly established, but a complete genetic analysis is yet to be completed. Recent findings reveal that a Drosophila cardiac muscle type known as ventral longitudinal muscles (VLMs) can be utilized as a genetic model for SHF development, since Drosophila orthologs of the mammalian factors Tbx1 and Islet1 are required for VLM formation. We used a UAS RNAi assay utilizing a VLM-specific Gal4 driver to knock down Drosophila orthologs of mammalian SHF determinants to confirm the use of Drosophila as an SHF model. A similar assay was performed to identify other genes involved in the contribution of heart defects in 22q besides TBX1. By discerning the phenotypes of the cardiac tubes and counting the longitudinal muscles present in the A3 and A4 regions of the heart field, it can be determined if any of the knocked-down genes create any cardiac abnormalities. Preliminary findings indicate that the genes sine oculis, moira, and CDC45L of the Digeorge (22q11.2) critical region, are all important in VLM development, and therefore warrant further study. Flies carrying knockdowns of these genes possess abnormal fiber counts that indicate they play a role in Drosophila cardiac formation and may be necessary for human cardiac formation. Findings generated from this research will be valuable in improving our understanding of, and potentially treating, 22q11.2 Deletion Syndrome and defects arising from SHF abnormalities.

1586S **Temporal effects of endosymbiont Wolbachia on the outcome of RNA viral infection in Drosophila melanogaster** Michael Rodwell[1], Casey Goltz[2], Brian Kmiecik[1], Madeeha Aziz[1], Lakbira Sheffield[3], Stanislava Chtarbanova[4] [1]University of Alabama, [2]Washington University School of Medicine, [3]BioGX, [4]Biology, University of Alabama

Wolbachia (*Wolb*) is an obligate, maternally-transmitted, intracellular bacterium known to infect ~50% of arthropods, including *Drosophila*. One aspect of *Wolbachia-Drosophila* interactions is a phenomenon called “pathogen blocking,” which corresponds to *Wolbachia*-mediated protection of *Drosophila* against pathogenic viral infections. The genetic and molecular mechanisms underlying this protection are not fully understood and are an ongoing area of investigation. Moreover, a gap in knowledge exists about the nature of the *Wolbachia-Drosophila* relationship as a function of host age. This is important to address as the aging process itself affects host physiology, including immunity to viral infections.

We have previously shown that aging impairs the ability of *Drosophila* to withstand the pathological consequences of infection (‘disease tolerance’ mechanism) with the RNA-containing Flock House Virus (FHV). Using 66 *Wolb*-free and 83 *Wolb*-positive lines from the *Drosophila* Genetic Reference Panel (DGRP), we demonstrate that the age-dependent survival of FHV infection is a continuous trait and is significantly modulated by the presence of *Wolb* independently of underlying lifespan. We established *Wolb*-free lines for several of the DGRP stocks in which older flies outlived younger flies after FHV infection and demonstrated that removing *Wolb* suppressed this phenotype. For one line, DGRP 320, using reverse transcription quantitative PCR (RT-qPCR), we show no significant changes in *Wolb 16S* gene expression, nor in FHV load between young and aged, *Wolb*-free and *Wolb*-positive hosts. Currently, we are confirming *Wolb* density in *Wolb*-positive samples for all experimental conditions and also conducting similar analysis in *Wolb*-positive DGRP lines in which older flies succumb faster of FHV infection. Our results indicate that *Wolb* possibly modulates disease tolerance to FHV with aging, independently of *Wolb* load.

1587S **Cellular and behavioral abnormalities in neurodegenerative models suppressed by altering heparan sulfate modifications in *Drosophila*** Nicholas Schultheis, Alyssa Connell, Scott Selleck BMMB, Pennsylvania State University

Mutations in *parkin* are the most common cause of early onset Parkinson’s disease (PD), and this gene plays a major role in mitophagy. Alterations in TDP-43 expression are highly associated with the progression of amyotrophic lateral sclerosis (ALS) and coincident mitochondrial dysfunction. This indicated the convergent biological importance of mitochondrial health in neurodegeneration. We identified heparan sulfate (HS) modified proteins as a class of cell surface and matrix molecules that regulate signaling and influence mitochondrial morphology and vesicular trafficking. By reducing expression of HS-constituent enzymes in *Drosophila* models, we achieved rescue of flight muscle degeneration and mitochondrial morphology in PD and pupal-stage lethality in ALS. Moreover, Alzheimer’s Disease (AD) is associated with mitochondrial and lipid catabolism dysfunction. Mutations in *presenilin/PSEN1* are the most common cause of familial, early onset AD. We examined the functional relationship between the cellular and behavioral pathology mediated by reduction in *presenilin* function and the activity of HS to modulate AD pathogenesis. In *Drosophila* and in human cultured cells, we explored the molecular characteristics of *presenilin* and HS downregulation through RNA sequencing analysis. Cell-type directed knockdown of *presenilin* in *Drosophila* revealed cellular abnormalities described in vertebrate models and humans. Reductions in mitochondrial size and number, deficits in autophagosome-to-lysosome trafficking, and lipid accumulation were observed. Conversely, knockdown of genes required for HS biosynthesis produced larger mitochondria, increased autophagy flux to the lysosome, and reduced levels of intracellular lipid. Cellular and behavioral abnormalities produced by *presenilin* knockdown were markedly suppressed by simultaneous reduction of HS biosynthetic encoding genes in the fruit fly. HS-mediated regulation of mitochondrial function, autophagy, and lipid metabolism was conserved in human cells.

Transcript analysis revealed a core group of SLC transporters and apolipoproteins that were significantly affected in opposite directions by decreases in expression of *presenilin* and HS. Simultaneous decrease in expression of *presenilin* and HS reverted this core group to near-wild type expression. Our work provides insight into the effects of HS interaction with *parkin*, TDP-43, and *presenilin*, and highlights the role SLC transporters may play in the progression of AD.

1588S **Parkin-null *Drosophila* vulnerable neuron mitochondrial hydrogen peroxide levels are reduced by manganese porphyrin compound** Amber N Juba[1], Riley Hamel[2], Petros P Keoseyan[2], T. Bucky Jones[3], Artak Tovmasyan[4], Lori M Buhlman[1] [1]Biomedical Sciences Program, Midwestern University, Glendale, [2]Arizona College of Osteopathic Medicine, Midwestern University, Glendale, [3]Anatomy, Midwestern University, Glendale, [4]Department of Translational Neuroscience, Ivy Brain Tumor Center, Barrow Neurological Institute

Identification of oxidative stress-initiating events may be key to developing successful therapeutic interventions for neurodegenerative disease. Excessive cellular reactive oxygen species (ROS) levels result from overproduction and/or decreased capacity of a variety of antioxidants. Elucidation of oxidative stress sources presents many obstacles and has wide-ranging implications for neurodegenerative diseases, including Parkinson's (PD). Homozygous loss-of-function parkin mutations cause a rare form of PD that shares hallmark pathophysiology with the idiopathic form. The *parkin* gene is conserved in *Drosophilae* (*park*), and flies have a dopaminergic brain region that is functionally homologous to the mammalian substantia nigra, which selectively degenerates in PD patients. We reported elevated protein oxidation in mitochondria of degenerating, but not non-degenerating, dopaminergic neurons in parkin-null *Drosophila*. We have also reported sustained elevated levels of hydrogen peroxide (H_2O_2) and transiently increased glutathione redox equilibrium (GRE) in vulnerable neuron mitochondria, but not in non-degenerating neuron mitochondria. Here we measured dopaminergic neuron mitochondrial H_2O_2 and GRE in parkin-null flies raised on food supplemented with MnTnBuOE-2-PyP^{5+}, a manganese porphyrin compound (MnP). MnPs are powerful superoxide dismutase mimetics that also catalyze protein cysteine oxidation and S-glutathionylation. MnTnBuOE-2-PyP^{5+} is being tested as a normal tissue radioprotector in clinical trials involving cancer patients undergoing radiotherapy. MnTnBuOE-2-PyP^{5+} improves developmental outcomes and decreases mitochondrial H_2O_2 in parkin-null fly dopaminergic neurons. Restoring redox imbalances may ameliorate parkin loss-of-function pathology and warrants study of MnTnBuOE-2-PyP^{5+} in additional neurodegenerative disease models toward use in clinical trials. There is currently no strategy for slowing PD progression. Despite the obvious implication of oxidative stress in PD models and patient tissue, antioxidant therapies have failed in clinical trial, perhaps because they lack critical target specificity or bioavailability.

1589S **Roles of Secreted Host Signals in Drosophila Tumor-Host Interactions** Kavya Adiga, David Bilder Molecular and Cell Biology, University of California, Berkeley

While tumor growth has been well-characterized, the interaction between a tumor and its host remains understudied. Tumors can have strong effects on the activity of distant organs, and these can be potent drivers for host lethality. Current work tends to focus on understanding the effects of tumor-secreted molecules on the host, but I am interested in how host tissues respond to a tumor. To study this, I use a simple cancer model in Drosophila. RNA sequencing reveals upregulated factors predicted to be secreted from host fat body (the major secretory organ; analogous to human fat and liver) in the presence of a tumor. Comparison of the response to tumor versus that to "chronic" sterile wounding reveals both shared and distinct molecules. Using a lifespan assay as a primary screen, I have identified candidates that when knocked down in the fat body affect cancer-induced host mortality. Some of these factors alter tumor burden and/or progression, while others appear to alter another aspect of host physiology. Further data on how these factors mechanistically impact tumor-host interactions will be presented.

1590S **Uncovering the mechanism of action of small molecules with neuroprotective potential in ALS using proteomics** Yash Kulkarni[1], Rachel A Allen[2], Deborah Cebular[1], Anne M Pruznack[1], Jon Njardarson[2], Daniela C Zarnescu[1] [1]Cellular and Molecular Physiology, Penn State College of Medicine, [2]Chemistry and Biochemistry, University of Arizona

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with a prevalence rate of 7 cases per 100,000 people worldwide. Cytoplasmic inclusions of hyperphosphorylated and ubiquitinated TAR DNA binding protein (TDP-43) are found in 97% of ALS cases and thus represent a major pathological hallmark of ALS. Although a variety of therapeutic approaches have been designed for ALS or directed against TDP-43 using cells and animal models, success in slowing down the disease has been limited. To address this, we performed an in vivo phenotypic screen aimed at mitigating TDP-43 associated locomotor and survival deficits in a Drosophila model of ALS based on overexpression of wild-type or mutant TDP-43 in motor neurons. Using this strategy, we identified three novel compounds from unique chemistry collections. Through a hit expansion campaign focused on one of these small molecules, we developed over 50 analogs, which afforded a new lead compound with improved activity (RAA-47). Next, we set out to identify the mechanism of action for RAA-47 using proteomics. To avoid the developmental lethality caused by pan-neuronal expression of TDP-43 we used the Elav Gene Switch GAL4 driver

to conditionally express TDP-43 in adults while administering RAA-47 or DMSO as vehicle control. Preliminary results show alterations in mitochondrial and metabolic proteins that we are currently validating. We are also using biotinylated analogs in conjunction with streptavidin pull-downs and mass spectrometry to identify the molecular target(s) of RAA-47. We will present our progress on identifying the molecular target and pathways modified by RAA-47 in flies and human cell lines with TDP-43 proteinopathy.

1591S **A Drosophila model of dementia based on C9orf72 hexanucleotide repeat expansion (HRE) exhibits age dependent axonal degeneration and FTD relevant behavioral phenotypes** Megan A Brennan[1], Brijesh Chauhan[1], Sharon Shaju[2], Keating R Godfrey[3], Daniela C Zarnescu[1] [1]Molecular and Cellular Physiology, Pennsylvania State University College of Medicine, [2]Juniata, [3]University of Florida

G4C2 Hexanucleotide Repeat Expansions (HREs) within the C9orf72 gene can cause the most common inherited forms of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). HREs are transcribed into RNA, transported into the cytoplasm, and translated into DiPeptide Repeats (DPRs) via Repeat Associated Non-AUG (RAN) translation. Both sense and antisense strands can be translated in multiple reading frames, creating several types of DPRs, which just like the G4C2 RNA are linked to neuronal toxicity. The HRE RNA and DPRs can cause neuronal degeneration over time, leading to ALS, FTD, or co-morbid ALS-FTD. In patients with FTD, disinhibition in behavior, becoming emotionally or socially isolated, dementia, and personality changes are common symptoms. Certain variants of FTD have overlapping phenotypes with ALS including brain degeneration, reduced lifespan, and loss of motor coordination. Several Drosophila circuits have similar functionality to human circuits including the Mushroom Body (MB), which regulates working memory and sleep among other behaviors. Here we characterize a novel model of dementia based on C9orf72 expressing either 12 or 44 G4C2 HRs in a subset of Kenyon cells comprising the MB circuit. Morphological analyses using confocal microscopy show evidence of age dependent axonal thinning in flies expressing HRs compared to w1118 controls. We could also detect age and sex associated changes in GFP-tagged DPRs (GR-GFP) generated via RAN translation, that correlate with axonal degeneration. Longevity analyses show that the presence of HRs decreases lifespan compared to w1118 controls, with male flies being more severely affected by the presence of G4C2 repeats than females. Using Drosophila Activity Monitors (DAMs) we also find that both HRs and sex play a role in altering activity and sleep behaviors even in young flies. Current experiments include using a y-maze assay to evaluate age dependent changes in overall movement and alternation, which is considered a measure of working memory. In summary, this model recapitulates several FTD relevant phenotypes which provide a novel model for studying C9orf72-FTD that can be used for future in vivo screening of genetic and small molecule modifiers of neurodegenerative disorders.

1592S **Uncovering the protective role of glycolysis in ALS using genetic and pharmacological approaches in *Drosophila* models of TDP-43 proteinopathy** Sara Gherardi[1], Suvithanandhini C Loganathan[2], Malajah Johnson[3], Daniela C Zarnescu[4] [1]Molecular and Cellular Physiology, The Pennsylvania State University College of Medicine, [2]University of Arizona, [3]The Pennsylvania State University College of Medicine, [4]Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine

Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease. TAR DNA binding protein (TDP-43) is a DNA/RNA binding protein which typically resides in the nucleus, but mislocalizes to the cytoplasm and forms inclusions, consequentially leading to a nuclear loss of function and a cytoplasmic gain of function. Regardless of etiology, 97% of patients exhibit TDP-43 cytoplasmic inclusions, referred to as TDP-43 proteinopathy. Recent studies have uncovered evidence of metabolic dysregulation in patients and animal models exhibiting TDP-43 proteinopathy; however, this remains poorly understood. Platelet phosphofructokinase (PFK) is the most abundant member of the PFK family of proteins in the nervous system, and catalyzes one of the rate limiting steps in glycolysis. PFK has 2 ATP binding sites, with one acting as an allosteric modulator and the other as a catalytic site. Our lab has previously shown that overexpression of PFK can mitigate ALS-related phenotypes. The goal of this study is to investigate the mechanism of action by which PFK overexpression is protective in *Drosophila* models of TDP-43 proteinopathy, and to identify other key enzymes in glycolysis that can mitigate these phenotypes. To determine whether the catalytic activity of PFK is required for neuroprotection, we generated transgenic flies that can overexpress a variant of PFK harboring a mutation in its ATP binding site required for catalysis (PFK^{ATPbs}). Using the D42 GAL4 driver, we found that overexpression of wild-type PFK (PFK^{WT}) in motor neurons mitigates $TDP\text{-}43^{WT/G298S}$ dependent locomotor deficits. There was also a rescue in survival when PFK^{WT} was overexpressed in the context of $TDP\text{-}43^{WT/G298S}$; however, no rescue was observed with PFK^{ATPbs}, indicating the need for an enzymatically functional PFK to improve TDP-43 dependent phenotypes. We are currently using confocal microscopy to analyze PFK puncta in the ventral nerve cord of third instar larvae. We are also taking pharmacological approaches to identify other enzymes in glycolysis that could mitigate ALS-related phenotypes. Administration of terazosin, a phosphoglycerate kinase 1 agonist, showed a rescue in survival in flies overexpressing $TDP\text{-}43^{G298S}$ in motor neurons. These results identify PFK and glycolysis as a potential therapeutic target and exploring its mechanistic contribution to ALS could lead to the development of novel compounds or diets which can help alleviate ALS patient symptoms.

1593S **Neuronal hyperexcitability and tactile hypersensitivity in a *Drosophila* larvae model of Neurofibromatosis 1** Anneke R Knauss[1], Seth M Tomchik[1,2,3] [1]Neuroscience and Pharmacology, University of Iowa, [2]Department of Pediatrics, University of Iowa, [3]Iowa Neuroscience Institute, University of Iowa

Neurofibromatosis type 1 (NF-1) is an inherited monogenetic disorder caused by the mutation and subsequent dysfunction of the neurofibromin 1 gene (*NF1*), a known tumor suppressor that encodes the neurofibromin protein (Nf1). In humans, the disease is diagnosed early in development and is characterized by the formation of tumors in the nervous system, altered metabolism, decreased stature, and decreased life expectancy. There are no treatments for NF-1, largely due to the complex downstream effects of the dysfunctional protein. The fruit fly, *Drosophila melanogaster,* is a powerful model for studying this disease; in *Drosophila*, the Nf1 protein is ~60% homologous to the human Nf1 protein at the amino acid level. In this well-established animal model, *nf1* mutants display significant decreases in both body size and lifespan. Increases in overall grooming behavior and metabolic rate are also observed. Interestingly, the grooming phenotype relies on proper Nf1 function during the pupal stage of *Drosophila* development; knockdown of Nf1 only in the adult stage does not drive excessive grooming. In *Drosophila* larvae, the reduction of functioning Nf1 induces neuronal hyperexcitability and tactile hypersensitivity; however, the mechanisms for these findings are currently unknown. Using *Drosophila* larvae, we are investigating the neuronal circuitry and cellular mechanisms underlying the developmental necessity of Nf1. Larvae exhibit a sophisticated locomotion repertoire in response to nociceptive stimuli to protect themselves from perceived danger. Previous studies and preliminary data from our lab show an increase in stereotyped escape behavior from *nf1* mutant larvae in response to nociceptive stimulation, a response likely propagated by sensory neurons. Using this behavioral paradigm, we plan to identify the optimal candidate neuronal population for Nf1 study using *Drosophila's* sophisticated genetic toolkit.

1594S **Molecular Dissection of Non-Olfactory Roles of an Odorant Binding Protein** Benjamin Soto, Nichole A. Broderick Biology, Johns Hopkins University

Odorant binding proteins (Obps) are canonically associated with functions in olfaction. Obps are functionally conserved from invertebrates to mammals, but are most well studied in insects. Loss of function studies of several Obps in various models demonstrate that some Obps play no, or minor, roles in olfaction, but have other impacts on host physiology. We previously reported that siRNA knockdown of the Tsetse fly Obp6 and knockout of its ortholog in Drosophila, Obp28a, led to reduced wound healing due to impacts on melanization. As such, these flies had reduced crystal cell populations, phenoloxidase activity, and expression of the crystal cell differentiation factor lozenge. Importantly, diminution of crystal cells is observed in both larvae and adults of both fly species. Collectively, this illustrates that Obp6/28a: a) are functionally conserved across taxa, b) perform functions outside of olfaction, and c) exert their impacts early in development, but with lasting effects across life stages. We will further discuss the mechanism by which Obp28a mitigates wound healing and its additional impacts on host physiology, including Obp28a patterns of expression to understand the epistasis of Obp28a in crystal cell development. Altogether, this work will shed light on the role of Obps in insect physiology outside of their previously hypothesized roles in olfaction.

1595S **Spenito-dependent metabolic sexual dimorphism intrinsic to fat storage cells** Arely V Diaz[1], Daniel Stephenson[2], Travis Nemkov[2], Angelo D'Alessandro[2], Tânia Reis[1] [1]Department of Medicine, University of Colorado Anschutz, [2]Department of Biochemistry and Molecular Genetics, University of Colorado Denver

Metabolism in males and females is fundamentally different. Differences are usually linked to sexual reproduction, with circulating signals (e.g., hormones) playing major roles. In contrast, sex differences prior to sexual maturity and intrinsic to individual metabolic tissues are less understood. We analyzed *Drosophila melanogaster* larvae and found that males store more fat than females, the opposite of the sexual dimorphism in adults. We show that Spenito (Nito), a conserved RNA-binding protein and regulator of sex determination, is required for the metabolic differences intrinsic to the major fat storage tissue, including many differences in the expression of metabolic genes. Nito knockdown specifically in the fat body (FB) abolished fat differences between males and females. We further show that Nito is required for sex-specific expression of the regulator of sex determination, Sex-lethal (Sxl). "Feminization" of fat storage cells via tissue-specific overexpression of the female determinant isoform of Transformer (TraF) in male and female FBs made larvae lean and reduced the fat differences between males and females. Correlating with these reduced fat differences, and consistent with inducing female-like expression in the FB, TraF overexpression shifted the male FB expression of metabolic genes to a female-like expression pattern. At the molecular level, Nito is a component of the N-methyladenosine (m^6A) methyltransferase complex where it plays a role in alternative splicing via m^6A-RNA modification. To test an m^6A-dependent modification requirement for metabolic dimorphism, we knocked down other members of the complex and found that Virilizer, fl(2)d, and Mettl14 are also required for metabolic differences between males and females. We are currently exploring the contributions of different diets to Nito- and m^6A-mediated metabolic dimorphism.

1596S **Understanding the long-term impacts of early-life exercise** Heidi M Johnson[1], Nicole Riddle[2] [1]Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

Exercise is a powerful and accessible therapeutic strategy for preventing or managing many disease conditions such as diabetes and cardiac dysfunction. However, exercise response demonstrates variation between individuals associated with sex and genetic makeup. While it is generally agreed that exercise has benefits as part of a healthy lifestyle, the relationship between exercise and lifespan is poorly understood. Here, we investigate the lifetime impacts of exercise using *Drosophila melanogaster*. We measured lifespan, activity level, body condition, physical ability, and reproductive health to determine how exercise treatments affect resource allocation to activity, somatic maintenance, and reproduction. We found that the impact of exercise on these measures of health is complex, with most responses depending on the interactions of sex, genotype, and treatment. Interestingly, a longer treatment (20 days) did not elicit consistently stronger effects than a shorter exercise treatment (5 days). However, the 20-day exercise treatment was associated with decreased climbing performance compared to controls up to several weeks after treatment ended, suggesting that long exercise treatments have the potential to be detrimental. Together, these results reveal the complexity of factors controlling exercise response, confirm the importance of genotype and sex, and highlight the importance of designing personalized exercise treatments to obtain optimal outcomes.

1597S **Single-cell analysis reveals immune activation in the tumor-host interaction** Hong-Cun Bao Tulane University

Cancer is one of the most feared diseases in our nation; however, the mechanism by which cancer actually takes a life remains to be fully explored. Studies on the mammalian immune system have developed several tools that revolutionized cancer treatment. Nevertheless, the complexity of the mammalian system remains a formidable challenge for researchers. The fruit fly is a relatively simple model organism and provides an opportunity to avoid such complexity. Here we take advantage of fly's simplicity to study tumor-host interactions and how tumors kill files.

We use NICD overexpression salivary gland tumor injection model to study tumor–host interactions. Technically, we allograft the NICD tumor from larval salivary gland into the abdomen of an adult fly host, allowing the tumor to grow in the host for 10 days. Subsequently, we dissect the adult, take out the tumor, and cut it into pieces small enough to perform another round of allograft. This allografting process can sustain the tumor for a maximum of 2 years.

During this period, we collect both the host and remaining tumor tissue for bulk-RNA sequencing or single-cell sequencing. Through gene set enrichment analysis of host bulk-RNA sequencing, we identify innate immune response as the top enriched term. Furthermore, through single-cell analysis of the tumor tissue, we identify two groups of cells that are not tumor cells but hemocytes (marked by Hml and He) and fat body cells (marked by Apolpp and Yp1) from the tumor host. Further data analysis reveals the expression of various immune response genes, such as antimicrobial peptides (AMPS) and peptidoglycan recognition proteins (PGRPs), in these two types of cells, while the tumor cells do not exhibit such expression. In detail, fat body cells exhibit high levels of Attacins, Diptericins, Bomanins, and immune-induced molecules, whereas Cecropins are predominantly expressed in hemocytes. As for the PGRPs, PGRP-SB1, PGRP-SC2, and PGRP-SD are primarily expressed in fat body cells, while Hemocytes express PGRP-LF.

In conclusion, the IMD pathways were activated in the tumor host, as confirmed by DptA-lacZ staining.

1598S **MICOS Complex Effect on Mitochondrial Dysfunction During Aging** Victor Knowles[1], Stephanie Uzordinma Awuzie[1], Mikesha Carter[1], Laura Galvan[1], Madison Raub[1], Angie del Barco[1], Antentor Hinton[2], Blake Riggs[1] [1]San Francisco State University, [2]Vanderbilt University

The cristae are important for driving oxidative phosphorylation in the electron transport chain to generate ATP. The cristae's size and shape are believed to be regulated by the mitochondrial contact site and cristae organizing system (MICOS). MICOS is a protein complex composed of subunits found at the inner membrane of the mitochondria. However, it is unknown how the MICOS complex regulates and maintains the cristae over the life of the mitochondria and if defects in MICOS function contribute to cellular aging. Here, we hypothesize that loss of MICOS complex function over time contributes to mitochondrial defects leading to cellular dysfunction found during aging. To test this hypothesis, we employed an aging assay using Drosophila melanogaster with loss of the components of the MICOS complex. Using the GAL4/UAS system, we used RNAi to inhibit Mitofilin and CHCHD3 in the MICOS complex to measure a decrease in lifespan. Then the flies were transferred to a different vial regularly and dead flies in each vial were recorded. Our data did not reveal a significant difference in lifespan in CHCHD3 and Mitofilin in comparison to our wildtype, however, we did see a longer survivability rate in females as compared to males in Mitofilin and CHCHD3 RNAi, contrary to our control. Quantitative PCR was utilized to measure transcript levels of MICOS subunits and mitochondrial regularity genes across the aging of Drosophila, from the embryonic stage to 75-day-old flies. Our results revealed a small decrease in CHCHD3 and Mitofilin transcription levels in Drosophila aged from embryo to 75-day-old flies. Going forward we plan to conduct qPCR on MICOS RNAi flies to determine the efficiency of mRNA

transcription suppression through the lifespan of Drosophila. We will use immunohistochemistry and confocal microscopy to detect levels of oxidative stress using 8-OHdG as a biomarker. Then we will use Mito-GFP with various MICOS components to image morphology and structure impacts in the heart and skeletal muscle cells of Drosophila.

1599S **Metabolomics and Lipidomics Studies reveal altered nutrient metabolism in the non-obese Nepl15 mutant flies** Surya Jyoti J Banerjee[1], Shahira H Arzoo[1], Soumyadip Kundu[2] [1]Biological Sciences, Texas Tech University, [2]Cell Biology, Mimetas

The Drosophila specific non-catalytic Neprilysin like 15 (Nepl15) is a major regulator of glycogen and glycerolipids storage. Nepl15 knock-out (*Nepl15^{KO}*) adult flies consume same amount of foods, however, the mutant adult male flies store significantly low amounts of lipids and glycogen, whereas mutant adult female flies store slightly less lipids and higher amount of glycogen compared to the control flies. Accordingly, mutant flies have anti-obesity features like extended life-span and ability to remain active at older age. Thus, it is important to take a deeper look at the nutrient metabolic pathways to understand alterations in the cellular mechanisms in these mutant flies. Therefore, we have performed analysis of the primary metabolomics and lipidomics using age-matched mutant and control adult male and female flies. Our preliminary analysis using the Metaboanalyst platform reveals that primary metabolites and lipids are different between the two sexes in the normal, and mutant adult fly groups using the Principal Component Analysis (PCA). Partial differences in the primary metabolites and the lipids are seen between the adult *Nepl15^{KO}* and control male flies, and the adult *Nepl15^{KO}* and control female flies in PCA. However, complete separations of primary metabolites and lipids between the *Nepl15^{KO}* and control male and female flies are evident in sPLSDA and oPLSDA analyses. While considering 1.5 folds change in the *Nepl15^{KO}* flies relative to the controls, we found that the *Nepl15^{KO}* adult males have 20 upregulated and 4 downregulated primary metabolites, and 48 upregulated and 193 downregulated lipids; while the *Nepl15^{KO}* adult females have 17 upregulated and 20 downregulated primary metabolites, and 47 upregulated and 81 downregulated lipids. Our analyses identified changes in the amino acid metabolism pathways between the mutant and control male and female flies. Thus, our data indicates an overall change in the nutrient metabolic pathways between the *Nepl15^{KO}* and control adult flies of each sex. Additional analysis of our data can provide detailed information about the metabolic pathways that are controlled by Nepl15 in future. This information ultimately will be useful to find new strategies to regulate obesity and other metabolic disorders.

1600S **Progress in Development of an Advanced Cancer Cachexia Model** Sofiane GANA, Mardelle Atkins, Morgan Marsh, Oluwapelumi Soyebo, Grace Stegemoller, Ellen Thompson, Logan McDowell Biology, Sam Houston State University

Cachexia is a complex wasting disorder which results in metabolic derangement and rapid loss of body muscle. It affects up to 80% of cancer patients and may be the direct cause of mortality for around 20% of all cancer patients. Yet, diagnostics and therapeutics for this devastating condition are extremely limited. Prior work in murine models have had limited success in translating their findings to the clinic. Thus, we have developed a cachexia model in Drosophila that recapitulates key features of cachexia, but in a more rapid and scalable system. We will present our current characterization of this model as well as novel insights gained.

1601S **Increased dopaminergic release confers neuroprotection against a *Drosophila* model of sporadic Parkinson's disease.** Angeline Claudia Atheby[1], Katarzyna Rosikon[1], Hakeem Lawal[1], Angeline Claudia Atheby[2] [1]Delaware State University, [2]Biological Science, Delaware State University

Parkinson's disease (PD) is a debilitating neurodegenerative disease characterized pathologically by the loss of dopaminergic neurons of the substantia nigra pars compacta. Decades of research have established key environmental and genetic factors as contributors to its etiology although the precise cause of most cases the disease remains unknown. Moreover, despite the advances in our understanding of the possible causes of PD, a viable treatment remains elusive. Rotenone, a potent laboratory model for sporadic PD has been used to uncover important insights into the etiology of the disease. We are interested in developing new disease-modifying therapeutic strategies against PD. Our approach of choice is to test a neuroprotective strategy based on increasing in the physiological release of dopamine (DA). To accomplish this goal, we are testing the neuroprotective capability of the small molecule dacarbazine (which we identified in a previous pharmacological screen) and its structural derivative, 5-Amino-4-imidazolecarboxamide (AICA). Both compounds have been reported previously to increase synaptic activity by potentiating the function of the vesicular monoamine transporter (VMAT, which facilitates the packaging and transport of DA into synaptic vesicles for exocytotic release). In this project, we investigated whether both compounds are capable of conferring organismal and/or neuroprotection against rotenone toxicity. We report that dacarbazine confers a small but reproducible protection against organismal toxicity induced by rotenone exposure in both male and female *Drosophila.* These results are all the more remarkable given that dacarbazine is a chemotherapeutic drug with a toxic potential of its own. Crucially, we report for the first time, that consistent with its published role as a VMAT-dependent drug, AICA protects dopamine (DA) neurons against rotenone-induced neuronal toxicity in an assay in which we combined both a

pesticide (rotenone) and age as risk factors for PD. We also present preliminary results of the effects of both compounds on rotenone-induced increase in oxidative stress. Together, these findings identify a promising new compound against PD and validate the importance of a physiological increase DA release as a viable therapeutics strategy against the disease.

1602S **The Drosophila Covid Resource (DCR) is used to reveal key functional interactions between SARS-CoV-2 NSP8 and host factors.** Margot Mel de Fontenay[1], Annabel Guichard[1], Daniel Bressen[2], Ketta Sneider[1], Shenzhao Lu[3], Oguz Kanca[3], Sara Sanz Juste[4], Shinya Yamamoto[3], Hugo Bellen[3], Ethan Bier[1] [1]University of California San Diego, [2]Universidade Federal do Rio de Janeiro, [3]Baylor College of Medicine, [4]The University of Texas MD Anderson Cancer Center

SARS-CoV-2 infections still represent a significant public health concern, while long Covid remains poorly understood. Development of effective therapies against Covid and long Covid relies on mechanistic knowledge of virus-host interface. Numerous host proteins have been shown to physically bind to individual viral factors, but the functional meaning of such interactions is less understood. We present a comprehensive Drosophila COVID-19 resource (DCR), which consists of publicly available lines for conditional tissue-specific expression of all SARS-CoV-2 encoded proteins, UAS-human cDNA transgenic lines encoding established host-viral interacting factors, and GAL4 insertion lines disrupting fly homologs of SARS-CoV-2 human interacting proteins. We demonstrate how of the DCR can be used to functionally assess SARS-CoV-2 genes and candidate human binding partners. We show that NSP8 engages in strong genetic interactions with several human candidates, most prominently with the ATE1 arginyltransferase to induce actin arginylation and cytoskeletal disorganization, and that two ATE1 inhibitors can reverse NSP8 phenotypes. The DCR enables parallel global-scale functional analysis of SARS-CoV-2 components in various tissues relevant to Covid pathologies.

1. Guichard, A., S . Lu, O. Kanca, D. Bressan, Y. Huang, M. Ma, S. Sanz Juste, J.C. Andrews, K.L. Jay, M. Sneider, R. Schwartz, M.C. Huang, D. Bei, H. Pan, L. Ma, W. Lin, A. Auradkar, P. Bhagwat, S. Park, K.H. Wan, … Bier, E. (2023). A comprehensive Drosophila resource to identify key functional interactions between SARS-CoV-2 factors and host proteins. Cell reports, 42(8), 112842. Advance online publication. https://doi.org/10.1016/j.celrep.2023.112842 PMID: 37480566

1603S **Single-Cell Insights into Aging, Aging-related Disease, and Longevity in Drosophila** Tzu-Chiao Lu[1,2], Ye-Jin Park[1,2,3,4], Hugo J. Bellen[2,3,4], Hongjie Li[1,2] [1]Huffington Center on Aging, Baylor College of Medicine, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, [3]Program in Development, Disease Models and Therapeutics, Baylor College of Medicine, [4]Jan and Dan Duncan Neurological Research Institute, Baylor College of Medicine

Aging, a complex biological process, has profound implications for healthspan and the progression of age-related diseases. In this study, we present a comprehensive analysis of Drosophila single-nuclei datasets, encompassing the Aging, Alzheimer's Disease, and Rapamycin Fly Cell Atlases. Our focus is to elucidate the intricacies of cellular aging, unravel the pathogenesis of age-related diseases, and decipher the mechanisms underlying longevity. We have refined cell type annotations to capture the subtle nuances of aging processes and the rejuvenating effects of rapamycin, a compound renowned for its lifespan-extending properties. Our integrative approach combines data across multiple dimensions—age, sex, genotype, and rapamycin treatment—to construct a detailed view of the cellular aging landscape. Additionally, we have developed a user-friendly, interactive visualization platform, enhancing the accessibility of these complex datasets for a broad range of scientific inquiries. Through this integrative endeavor, we aim to deepen our understanding of the molecular and cellular dynamics of aging and longevity, offering valuable insights into Drosophila aging research.

1604S **Identifying Candidate Genes and Genetic Networks that Influence the Age-specific Ability to Clear an Infection Using a Genome Wide Association Study (GWAS)** Shonda Campbell University of Maryland Baltimore County

The innate immune response is an evolutionarily conserved process that is essential for survival in multicellular organisms. However, as individuals age, immune functions begin to decline, a phenomenon known as immunosenescence, which can vary greatly among individuals. This variation has a genetic component yet the genes that contribute to this variation are widely unknown. Here, we used 183 genetically distinct genotypes of the *Drosophila* Genetic Reference panel (DGRP) to assess their ability to clear an infection at one and five weeks of age, and then carried out a genome-wide association study (GWAS) to identify candidate genes that contribute to differences in immune responses among genotypes at each age. We also carried out a genetic network analysis using the candidate genes identified in the GWAS to identify signaling pathways that contribute to age-dependent variation in immune function. We found that clearance ability varied greatly among genotypes, but on average, the ability to clear an infection declined by 70% with age. We identified 153 genes significantly associated with clearance at 1 week of age, 52 genes significant at 5 weeks of age, and only two genes, *Cc2d2a* and *lipophorin receptor 1* (*LpR1),* significant at both ages. Of the identified candidate genes, 53 were mapped to genetic networks. Gene Ontology Analysis of genes in the networks implicate genes encoding proteins involved in cell adhesion, migration and those associated with the plasma membrane as important contributors to variation in immunity. Network analysis also implicated several

signaling pathways as contributing to genetically based differences in the ability to clear infection. Our results indicate that the genetic basis of naturally occurring variation in immune function changes with age. Future work will focus on how these genes influence immune function to better understand the molecular mechanisms that give rise to age-dependent genetic effects, which will be important for understanding the genetic basis of senescence.

1605S **The effects of phytocannabinoids on *Drosophila melanogaster* midgut, lifespan and locomotor activity** Sandra C Illescas, Alyssa M Vidal, Cristian Rodriguez, Mariano Loza-Coll Biology, California State University, Northridge

Phytocannabinoids, compounds found in the *Cannabis sativa* plant, can activate the canonical CB1/2 endocannabinoid system in mammals, and have garnered increasing attention for their potential health benefits. While numerous studies have explored their effects on mammalian systems, limited research exists on the impact of phytocannabinoids on invertebrate models. *Drosophila melanogaster* does not have CB1/2 receptors, but offers a valuable platform to investigate potential physiological effects of phytocannabinoids via CB1/2-independent, yet well conserved molecular pathways.

Here we present preliminary work aimed at assessing the influence of broad spectrum phytocannabinoid extracts containing cannabidiol, cannabigerol, delta-9-tetrahydrocannabinol and others on *D. melanogaster* midgut homeostasis, locomotor behavior and lifespan. Adult flies carrying an *esg*-Gal4ts>UAS-*gfp* were exposed to phytocannabinoids via dietary supplementation, and immunofluorescence microscopy followed by automated CellProfiler analysis revealed morphological changes in *D. melanogaster* midguts, including alterations in progenitor morphology and the relative proportion of enteroendocrine cells in the intestinal epithelium. We also complemented our analysis of intestinal homeostasis with a standard lifespan assay, as well as climbing assays to determine any differences in locomotor activity. Lastly, we also analyzed whether exposure to phytocannabinoids, which are known to have anti-inflammatory effects in mammals, could protect fly intestinal epithelia from the oxidative stress caused by exposure to Paraquat.

Our preliminary findings suggest that phytocannabinoids have complex effects on midgut morphology, which correlate with broader physiological effects. Further work will be aimed at experimentally manipulating genetic pathways known to serve as non-canonical targets of phyto- and endo-cannabinoids in mammals, and thus explore their potential roles in mediating their effects on flies. Our work will hopefully unveil alternative yet conserved mechanisms that might better inform their administration in the context of human health.

1606S **RNA-Seq analysis starvation selected Drosophila melanogaster** Aavash Adhikari, Allen Gibbs School of Life Sciences, University of Nevada Las Vegas

Obesity is a common, serious and costly disease with 41.9% prevalence in the US. The heritability estimate of obesity is up to 70% indicating a huge role of genetics as its causal factor. Although we know much about the genetics behind obesity, understanding it at the transcriptomic and proteomic levels will further give us some insights into its molecular physiology. Drosophila melanogaster, which has been selected for starvation resistance, develops slower, is larger in size, and has higher lipid content than normal-fed flies. They also have altered physiologies like cardiac dysfunction, reduced sleep performance, and reduced fecundity. We are using those flies as a model for understanding human obesity by analyzing their transcriptome and proteome at the third instar larvae, as previous research has shown that this is the key stage differentiating between starvation-selected and control flies.

Transcriptomic analysis revealed genes that were differentially expressed between selected and control populations. Out of 15094 with nonzero total read count (adjusted p-value < 0.01), 2983 (20%) genes were upregulated and 2029(13%) genes were downregulated. Gene set enrichment analysis revealed functions like ribosome biogenesis, fatty acid metabolic process, carboxylic acid and oxoacid metabolic process were activated in starvation selection flies. In contrast, developmental processes like imaginal disc eversion, leg disc development, and leg disc morphogenesis were suppressed. The results are consistent with our observation of longer development time for starvation-selected flies. We have obtained proteomic data from the same time frame and will analyze them to correlate with the transcriptome data. We hope combining the two data sets will further validate our results and give more information about the physiology of starvation-selected flies.

1607S **The autophagic stem cell loss promotes intestinal inflammation** Kang Han[1], Michael Boutros[2], Jun Zhou[1] [1]Hunan University, [2]German Cancer Research Center

The inflammatory bowel disease (IBD) is a complex and multifactorial intestinal disorder arising from gene-environment interactions. The genetic variants are major causes in IBD pathogenesis, however, only 30% of patients are diagnosed positive for known IBD causative genes. To identify additional IBD risk genes and unravel the disease mechanisms, we performed a large-scale in vivo CRISPR screen detecting IBD-like symptoms in *Drosophila* intestine. We discovered 105 of nearly 2000 tested

CRISPR knockouts develop IBD-like disorders, notably, 47 genes (45%) are previously known IBD causative genes in humans. We identified the ceramide synthase Schlank controls stem cell survival by regulating lipogenesis. The Schlank mutant exhibits a reduced lifespan with IBD-like disorders like intestinal barrier defect, inflammation, and diarrhea. Mechanistically, we showed Schlank knockouts displayed reduced lipid levels and impaired autophagosomal/mitochondria activity in stem cells, leading to stem cell death and organ failure, thereby causing intestinal inflammation. Inhibition of lipolysis and autophagy restores stem cell numbers and barrier function of the intestinal epithelium, prevents intestinal inflammation, and extends the organismic lifespan. We therefore uncover a previously underappreciated role for stem cell maintenance in the pathogenesis of IBD and demonstrate that stem cell revival could be the potential therapeutical strategy for IBD treatment.

1608S **Using Drosophila to Understand the Role of Polyamine Metabolism in Parkinson's Disease** Bedri Ranxhi[1], Zachary M Chbihi[1], Zoya Bangash[1], Zaina Qadri[1], Sokol V Todi[1], Peter A LeWitt[2], Wei-Ling Tsou[1] [1]Pharmacology, Wayne State University School of Medicine, [2]Neurology, Wayne State University School of Medicine

Polyamines play essential roles in various organisms, encompassing multiple physiological functions including cell growth, survival, and are involved in key biological processes, such as the synthesis of proteins and nucleic acids, stabilizing the structure of chromatin, regulating apoptosis, and safeguarding cells against oxidative damage. Polyamine metabolism has also been investigated for yielding disease-specific biomarkers. In a pilot study we identified increases in serum concentrations of three L-ornithine-derived polyamines (putrescine, spermine, and spermidine), each of which showed correlation to Parkinson's disease (PD) progression and its clinical subtypes. Considering the significant physiological roles that polyamines play in our body's functions and their tight homeostatic regulation, we further investigated if the insights gained from our biomarker discoveries could help us gain a better understanding of the biochemical aspects of neurodegeneration in diseases like Parkinson's, and others where proteinopathy is vastly prevalent. We conducted experiments in which we altered the way polyamines are processed in *Drosophila* models of synucleinopathy (knocking down critical polyamine interconversion enzymes). These models involve an excess of neuronal α-synuclein.

We observed substantial alterations in the lifespan and motility of *Drosophila* after suppressing key enzymes of polyamine metabolism (spermine synthase [SMS], spermidine/spermine N1-acetyltransferase 1 [SAT1], spermine oxidase [SMOX], or polyamine oxidase [PAOX]. As polyamine metabolism is vital for maintaining neuronal integrity, we investigated the functional involvement of each polyamine in neuronal homeostasis and their regulation with regard to α-synuclein. In addition, we investigate whether modifying particular polyamine enzymes in our fly model of α-synuclein overexpression would lead to a reduction in the extent of α-synuclein aggregation.Our analysis provides a deeper perspective into the underlying causes of neuronal and systemic changes in polyamine metabolism as it relates to α-synuclein proteinopathy. These findings may offer valuable guidance for designing clinical trials that focus on therapeutic interventions directed at the regulation of polyamine pathways.

1609S ***Drosophila* model of *de novo PHACTR1* variant demonstrates alterations in bristle structure and synapse function** Jonathan Andrews[1], Sharayu Jangam[1], Paige Hall[1], Lauren Briere[2], Shinya Yamamoto[3], David Sweetser[4], Michael Wangler[1] [1]Molecular and Human Genetics, Baylor College of Medicine, [2]Division of Genetics, Massachusetts General Hospital, [3]Baylor College of Medicine, [4]Medical Genetics, Massachusetts General Hospital

Phosphatase and Actin Regulator 1 (PHACTR1) is a member of a family of Protein Phosphatase 1 (PP1) binding proteins which defines the substrate specificity of the Phactr1/PP1 holoenzyme. PHACTR1 is broadly expressed within the central nervous system and is believed to play an important role in the regulation of actin cytoskeletal dynamics, PP1 activity, actin stress fiber formation, cell motility, and cell survival. Multiple *PHACTR1* variants have been previously associated with different human pathologies, including developmental and epileptic encephalopathy, West syndrome, myocardial infarction, melanoma growth, coronary artery disease, and cervical artery dissection. PHACTR1 is orthologous to *Drosophila CG32264*, which is highly expressed in the adult brain, anterior midgut primordium, larval midgut, and posterior midgut primordium. We have identified a human proband with a *de novo* duplication of exon 5 and parts of the surrounding introns who presented with global developmental delay, hypotonia, absent speech, seizures, behavioral problems, mild prominence of ventricles and sulci, and slight dysmorphism of the left hippocampus. Subsequently, we generated a *Drosophila* overexpression model of human PHACTR1 using the GAL4/UAS system to drive the expression of the human gene within specific tissues in the fly. As PHACTR1 is regulated by actin via the presence of RPEL domains, we sought to evaluate its effect on several actin rich or neurologically related structures in the fly model. Overexpression of either the reference or variant human cDNA via a Nubbin-Gal4 driver produced no changes within the wing tissues, and overexpression within the eye by GMR-Gal4 produced only minor changes in eye morphology. Ubiquitous overexpression of the putatively pathogenic variant using either an Actin, Tubulin, or Pannier-Gal4 driver was sufficient to induce forking or abnormal bends in bristles. These changes within the actin-rich bristle structures were not observed in animals overexpressing the reference human cDNA. Likewise, the ubiquitous overexpression of CG32264 showed no alterations in bristle morphology. Ubiquitous depletion of *CG32264* via

two different RNAi lines (*CG32264^{JF02975}* and *CG32264^{HMC05840}*) also failed to cause any changes in bristle morphology. However, the expression of *CG32264^{JF02975}*, but not *CG32264^{HMC05840}*, in eye tissues via GMR-Gal4 produced a rough-eye phenotype. An electroretinogram of the adult fly eye demonstrated significant changes in the on/off transients in animals expressing our PHACTR1 variant compared to both PHACRT1 reference and CG32264 expressing animals. Our initial assessment demonstrates that *Drosophila* is a suitable model for further exploration of *PHACTR1* variants and identifies that duplications of the 5th exon are putatively deleterious and may underly a novel disorder separate from those previously associated with *PHACTR1*.

1610S **Utilizing Trojan Exon Cassettes to observe altered neurodevelopmental and neurodegenerative phenotypes caused by *pex* mutations in *Drosophila*.** Vanessa A Gomez, Sharayu Jangam, Jonathan Andrews, Michael Wangler Molecular & Human Genetics, Baylor College of Medicine

Peroxisomal Biogenesis disorders (PBD) are a group of autosomal recessive disorders caused by loss-of-function mutations of one of the *PEX* genes responsible for peroxisomal formation. Impaired peroxisome assembly causes severe multisystemic failure with patient phenotypes ranging from epilepsy, feeding issues, biochemical abnormalities, and neurodegeneration. To study the neurodevelopmental and neurodegenerative phenotypes seen in patients, we moved to *Drosophila* as a model organism utilizing Trojan Exon Cassettes. Here, we generated a fly line in which the coding sequence of *pex2* has been replaced by the *Kozak-GAL4* promoter trap sequence. This cassette results in a simultaneous knock-out of the *pex2* and knock-in of a *GAL4* driver sequence. After crossing *pex2-Kozak^{GAL4}* lines to known *pex2* null alleles, lifespan analysis showed a significant difference in experimental flies than controls. These results are consistent with our known null alleles, suggesting that *pex2-Kozak^{GAL4}* is also a null. In parallel, we have generated UAS-human cDNA lines to assess the function of *PEX2* variants in *Drosophila* and compare variant function with human rescue. Human rescue will be done by replacing *pex2* with either reference or variant human transgenes and observing differences in phenotype that could suggest a *pex2*-specific effect. Utilizing the *pex2-Kozak^{GAL4}* and UAS-human cDNA lines, functional assays are underway, such as bang sensitivity and climbing assay, to observe and compare loss-of-function variant function with human rescue. Altogether, we hope to utilize these unique *Drosophila* lines to observe the neurodevelopmental and neurodegenerative phenotypes in *pex2* variants and better understand their pathogenicity.

1611S **The glycoprotein 5 of porcine reproductive and respiratory syndrome virus stimulates mitochondrial ROS to facilitate viral replication** wang jiang Henan Agricultural University

Viruses have evolved sophisticated mechanisms to manipulate host cell organelles to serve as niches for persistence and proliferation. In the present study, we aimed to investigate the role of cellular organelles in the replication of porcine reproductive and respiratory syndrome virus (PRRSV). We found that the morphology of mitochondria and the endoplasmic reticulum (ER) were both altered, and the contact between these two organelles was enhanced during PRRSV infection. By the overexpression of PRRSV-encoded open reading frames, we identified that only glycoprotein 5 (GP5) was essential for ER-mitochondria contact. Further investigation revealed that GP5 interacted with the ER inositol 1,4,5-triphosphate receptor (IP3R) and the mitochondrial voltage-dependent anion channel (VDAC1) to promote the Ca2+ efflux from ER into mitochondria. Excessive mitochondrial Ca2+ uptake resulted in mitochondrial dysfunction and substantial mitochondrial reactive oxygen species (mROS) production. Elevated mROS activated autophagy through the AMPK/mROR/ULK1 axis to facilitate PRRSV replication. GP5-induced mROS also triggered the NOD-like receptor family pyrin domain-containing protein 3 (NLRP3) inflammasome. Inhibition of autophagy augmented NLRP3 inflammasome activation and exhibited an anti-PRRSV effect, suggesting autophagy counteracted the NLRP3-mediated innate immune response. Overall, our findings highlighted the importance of cellular organelles in virus-host interactions and provided new insights into the complex interplay between virus replication and innate immune responses

1612S **A potential role of the gut microbiome in promoting age related alcohol tolerance in Drosophila** Patricia Pujols, Kerianalee Rivera, Imilce Rodríguez, Alfredo Ghezzi Biology, Universidad de Puerto Rico, Rio Piedras

Alcohol Use Disorder (AUD) is a medical condition characterized by a limited ability to stop or control alcohol use despite adverse social, occupational, or health consequences. In older adults, the habit of excessive alcohol consumption represents 20% between the ages 60 to 64 years and 11% over 65 years of age, according to the National Survey and Drug Use and Health. Aging can affect the response to alcohol in aspects of sensitivity and tolerance, which puts the elderly at greater risk. Thus, there is an urgent need to characterize the cellular and molecular effects of alcohol consumption in this aging population. Aging has twelve proposed hallmarks but of particular interest for our project is the altered intercellular communication between the animal genome and the microbiome leading to microbial dysbiosis. The human being is home to around 38 trillion bacteria and this collective commensal, symbiotic, and pathogenic microorganisms is known as the microbiota, the majority of which is found in the intestine. Our study aims to understand the effects of alcohol on the bidirectional interaction of the gut microbiome and the brain in aspects of tolerance and sensitivity in young and old animals. We are using *Drosophila*

melanogaster as a model organism as it has homology with humans in alcohol response, intestinal microbiome composition, and displays aging phenotypes. To measure age-related changes in alcohol behaviors, we exposed young (5 days) and old (5 0days) Canton-S female flies to 50% ethanol vapor. We then calculated the time they take to sedate. For sensitivity assays, we measured resistance to alcohol in a first exposure compared to a control group that is exposed to water. While for tolerance we calculated the difference between resistance from a second exposure generated by a previous one. We then measure the changes in the gut microbiome after one or two exposures to alcohol. To do this we dissected the intestine of treated flies and plated the homogenate in selective media for *Lactobacilli*, *Acetobacteria,* and *Enterobacteria* and measure abundance in Colony Forming Units (CFUs). Our data shows that there is an increase in sensitivity and tolerance in old flies. In young and old flies, we find that after one exposure to alcohol, all CFUs increase and then decrease during the second exposure. Young flies treated with antibiotics have a decrease in tolerance. Future studies are focused on finding the molecular mechanism used by specific bacterial species to influence sensitivity and tolerance in flies. We hope to elucidate the underlying causes of neuroadaptation possibly caused by changes in the gut microbiome and to further understand these changes in the aging context.

1613S **Regulation of intestinal stem cells and longevity by the nuclear envelope protein Klaroid (Koi)** Ithan Cano, Carlos Asturias, Courtney Frazier, Mariano Loza-Coll Biology, California State University, Northridge

Multicellular organisms often rely on resident adult stem cells (ASCs) to maintain tissue homeostasis. These oligopotent ASCs undergo self-renewing asymmetric divisions, by which they produce an identical copy of themselves and a differentiating sister that will give rise to any one of a limited range of terminally differentiated cells to replace those lost to injury, disease or normal tissue turnover. The activity of ASCs is governed by pleiotropic master regulator (MR) genes, which exert direct or indirect control over numerous downstream effector genes, most of which remain poorly understood.

Here we report preliminary findings on the role that the nuclear membrane protein Klaroid (Koi), an experimentally validated target of Escargot and dSTAT92E, two well-known and important MR genes in Drosophila intestinal stem cells (ISCs). Previous work by others established Koi's role in repairing double-strand DNA breaks in Drosophila cells in culture and its requirement for attachment of the nucleus to the cytoskeleton in the Drosophila eye. To our knowledge, no previous studies have specifically addressed Koi's role in ISCs.

Here we used ISC-specific genetic manipulations of Koi and immunofluorescence microscopy to determine the effect of over-expressing or knocking-down Koi on the number, morphology and relative abundance of different cell types in the intestinal epithelium, both in young flies and during aging. Since preliminary observations indicated that Koi overexpression might be protective against aging-induced intestinal dysplasia, we also conducted lifespan assays coupled with a Smurf assay to probe for intestinal barrier integrity. Flies with lower Koi expression in ISCs showed a significant shortening in lifespan compared to controls. Surprisingly, flies overexpressing Koi in ISCs had comparable survivorship to controls for approximately 35-40 days, but experienced a sudden and sharp mortality afterwards. We are currently trying to more carefully characterize the effect of Koi manipulations in ISC regulation, and thus identify potential links between the nuclear envelope, ISC biology and their secondary effects on lifespan.

1614S **The Stumble E3 Ligase Induces Interaction Between the SWI/SNF Component BRG1 and Beta-catenin to Promote Wnt Signaling** Kai Yuan[1], Sara Kassel[2], Nawat Bunnag[3], Ben Maines[3], Amber Adams[3], Zachary Spencer[3], Andres Lebensohn4[4], Rajat Rohatgi[5], David Robbins[6], Arminja Kettenbach[3], Ethan Lee[2], Yashi Ahmed[3] [1]Molecular and Systems Biology, Dartmouth College, [2]Vanderbilt University, [3]Dartmouth College, [4]National Cancer Institute, [5]Stanford University School of Medicine, [6]Georgetown University

The Wnt/β-catenin signal transduction pathway is an ancient pathway that regulates cell proliferation and differentiation during animal development. Inhibition or aberrant activation of Wnt/β-catenin signaling causes birth defects and several cancers, particularly colorectal cancer. Although the canonical Wnt pathway has been extensively studied, no FDA-approved drugs targeting the pathway exist. Here, starting with genome-wide forward genetic screens in human haploid cells, we identify an evolutionarily conserved ubiquitin ligase, Stumple, as a novel positive regulator of Wnt/β-catenin signaling. RNAi-mediated depletion and conditional CRISPR-mediated mutagenesis of Drosophila *Stumple* decreased Wnt/β-catenin signaling in multiple tissues. Genetic epistasis analysis showed that Stumple acts downstream of the β-catenin destruction complex. In the presence of Wnt, Stumple interacted with BRG1, a subunit of the SWI/SNF chromatin remodeling complex, and induced BRG1 ubiquitination. Stumple-induced ubiquitination did not affect BRG1 stability nor its association with SWI/SNF components. However, Stumple depletion decreased the interaction between BRG1 and β-catenin, the key transcriptional coactivator in the Wnt signaling pathway. Our findings support a model in which Stumple ubiquitylates BRG1 in the presence of Wnt and promotes its interaction with b-catenin, thereby bringing SWI/SNF to Wnt target genes. Our studies suggest a general mechanism by which cell signaling induces the interaction between BRG1 and pathway-specific transcription factors to

recruit SWI/SNF complexes to their appropriate target genes.

1615S **Protective Role of Exercise-responsive Genes against Chronic Muscle Disuse** Mousumee Khan, Jodi Protasiewicz, Li Tao, Charles Chung, Alyson Sujkowski, Robert J. Wessells Physiology, Wayne State University

Prolonged sedentary state due to medical conditions can cause chronic muscle disuse and lead to physical incapacity and poor quality of life. Here, we described a model of skeletal muscle disuse via enforced restraint utilizing *Drosophila melanogaster* to characterize the effects of sedentary behavior in flies. We found that restraint substantially reduced endurance, climbing speed and longevity in flies. Restrained flies accumulated significantly more polyubiquitin than unrestrained flies, indicating enhanced protein degradation in muscle of restrained flies. However, flies that were restrained for most of their life, but released five times a week to undergo an exercise program significantly ameliorated their endurance, climbing speed and muscle actin integrity accompanied by upregulation of autophagy and phosphorylated AKT (p-AKT). Lastly, we identified three exercise-responsive genes, *Sestrin* (*dSesn*), *Spargel* (*srl*) and *Iditarod* (*Idit*) whose overexpression in muscle provided significant protection against the effects of restraint. Proteins encoded by these genes represent targets that may be leveraged toward therapeutic options for patients with chronic inactivity.

1616S **Comparative Genomics Reveals DNA Elements that Regulate p38Kb in Aging and Stress** Brooke Allen[1], Alysia Vrailas-Mortimer[1,2,3] [1]Biological Sciences, Illinois State University, [2]Biochemistry and Biophysics, Oregon State University, [3]Linus Pauling Institute

Aging is complex process that results in the decline and deterioration of an organism's functions. A number of aging genes have been identified though how they are regulated in the context of aging is not well described. One such aging gene is the highly conserved p38 MAPK (p38Kb), which we have shown to extended lifespan and increased stress resistance when over-expressed in *Drosophila* muscle. Loss of p38Kb results in a shortened lifespan, premature locomotor dysfunction, and increased stress sensitivity. Though much is known how p38 MAPKs are regulated at the protein level, little is known about how p38 MAPKs are transcriptionally regulated. Therefore, we utilized a comparative genomics approach in which we analyzed the upstream region of the p38Kb gene across 22 sequenced species of Drosophila. We identified two conserved transcription factor binding sites in Drosophila species that were more resistant to oxidative stress and had increased levels of p38Kb protein in response to oxidative stress. One site is a predicted AP-1 biding site, while the other is a predicted lola-PT binding site. We found that over-expression of either AP-1 or lola-PT in D. melanogaster, which has both binding sites, was sufficient to induce p38Kb transcription under standard conditions. Only over-expression of lola-PT was able to induce p38Kb transcription under oxidative stress conditions. In order to determine if these binding sites play a functional role in p38Kb transcription during aging and stress, we have generated p38Kb null mutants that have a transgenic version of the upstream region and full gene sequence of the p38Kb gene with either have an intact promotor with both binding sites, a promotor with only the AP-1 site, a promotor with only the lola-PT site, or a promotor with both sites scrambled. Our preliminary findings suggest that the lola-PT site is required for stress response and viability, while the AP-1 site is required for lifespan.

1617S **Changes in immune signaling may mediate different outcomes between mild, repeated TBI and a single, severe TBI** Jorge A Garcia, Daniel Tulchinskiy, Kamden T Kuklinski, Doyinsola Ogunshola, Rebecca Delventhal Biology, Lake Forest College

Traumatic brain injuries (TBIs) are a common neurological disorder, affecting more than 1.5 million people nationwide and often cause hospitalization, long-term disability, and death. However, due to differences in age, sex, and other demographics, it is difficult to compare outcomes of different injury patterns amongst humans, especially because the characteristics of TBIs sustained are often highly variable. To study head injuries in a controlled manner, we used *Drosophila melanogaster* as a model organism to assess the effects of mild, repeated TBI (multi-day, MD) compared to a single, severe TBI (single-day, SD). We wanted to know if different patterns of TBI differ in short- and long-term outcomes. To determine this, we measured acute mortality, lifespan, and climbing. We found that flies given a SD TBI exhibited higher acute mortality, but the surviving flies showed a longer lifespan than flies given a MD TBI. Likewise, flies given a MD TBI showed worse long-term locomotor ability. We hypothesized that different immune responses to MD vs. SD TBI may mediate differences in short- and long-term outcomes. To test this, we measured expression of antimicrobial peptides (AMPs) 4 hours post-TBI and found that AMP expression increased after each day of the MD TBI and was eventually equivalent – if not greater – than expression four hours post-SD TBI. We are currently investigating long-term AMP expression post-TBI. We also measured acute mortality and lifespan of Imd and Toll mutant flies post-MD and SD TBI. We found that loss of Imd led to worse short- and long-term survival in both injury conditions, indicating that Imd signaling is protective in both injury types. Interestingly, while loss of Toll led to worse short-term survival after SD TBI, Toll mutants survived better after MD TBI. When we measured Toll mutants' lifespan post-MD and SD TBI, we saw that loss of Toll did not significantly change the flies' lifespan post-SD TBI but modestly increased the lifespan of flies post-MD TBI, suggesting that Toll signaling is detrimental for both short- and long-term outcomes from a MD

TBI. To understand cell-type specificity in Toll signaling, we are conducting glial and neuronal knockdowns of Toll and testing short- and long-term outcomes. Understanding differences in cellular immune responses to different types of TBI could enable the development of tailored treatments, improving outcomes.

1618S **Mutagenesis of *spastin*: a model for Hereditary Spastic Paraplegia and opportunity for a course-based undergraduate research experience (CURE)** Jerry Ye[1], Claudia Barreto[2], Myra Cai[2], Meera Gangasani[2], Sofia Guerrero[2], Cordelia Hume[2], Qizhou Jiang[1], Taranjit Saggu[2], Letian Wang[1], Nina T Sherwood[2], Emily F Ozdowski[2] [1]Biology, Duke Kunshan University, [2]Biology, Duke University

Locomotor dysfunction and progressive neurodegeneration are hallmarks of Autosomal Dominant-Hereditary Spastic Paraplegia (AD-HSP) patients. Mutations in multiple genes have been implicated, but approximately 40% of AD-HSP cases result from disruption of SPG4 / SPASTIN. The SPAST protein is a member of the AAA ATPase family and has been shown, across different cell types, to regulate the microtubule cytoskeleton. Given that the amino acid sequence is highly evolutionarily conserved, the microtubule interacting and trafficking (MIT) domain and the catalytic ATPase domain were also identified in *Drosophila melanogaster*. To characterize critical protein regions, we mutagenized fruit flies with the chemical, ethyl methane sulfonate (EMS). Point mutations generated in *Drosophila spastin* were isolated and sequenced, while individual fruit fly lines were tested for disruptions of viability and neuromuscular junction morphology. Here we report the results of these new mutations and the allelic series of severity that our data support. When compared to the genetic null, $spas^{5.75}$, this mutational analysis provided a readout of important amino acid residues and was accomplished within a single undergraduate semester. Each student was given an unidentified mutant line of *Drosophila* for characterization. For students, this type of project integrates different levels of analysis from basic genetics and cell biology to the application in human health. It is ideal for an exploratory upper-level lab course, a CURE (course-based undergraduate research experience), in which the students are given more independence and freedom of analysis than in introductory courses. Our course design included a breadth of laboratory techniques (molecular biology, animal husbandry, microdissection, immunofluorescence, and microscopy) and digital tools (DNA sequence analysis with SeqMan Ultra, image analysis with Image J/Fiji, graphing in R, and figure compilation in Adobe Photoshop). The assessments focused on data interpretation and visual science communication with a final poster presentation. The characterization of new mutations could be generalized to other easily screened phenotypes and the use of *Drosophila melanogaster* as the model organism makes the timeline of a single semester possible.

1619S **The prototypical TUB domain protein TUB bipartite transcription factor *Tub* regulates RNA trafficking** Tyler R. Henderson[1,2], Hamza Taufique[1,2], Rieko Niibori[1], Ulrich Braunschweig[3], Benjamin J. Blencowe[2,3], Sabine P. Cordes[1,2] [1]Lunenfeld-Tanenbaum Research Institute, [2]Department of Molecular Genetics, University of Toronto, [3]Terrence Donnelly Center for Cellular and Biomolecular Research

In mice and humans, mutations in the TUB bipartite transcription factor (*Tub*) gene cause maturity-onset obesity, insulin resistance, deafness and retinitis pigmentosa. Furthermore, mutations in *Tub* orthologues present within *Drosophila melanogaster* and *Caenorhabditis elegans* also cause metabolic and sensory deficits. TUB is the founding member of the TUB protein family; in mammals this includes TU-like proteins (Tulps) 1-4. Both Tub and Tulp proteins harbor a carboxy terminal nucleic acid binding domain - the TUB domain. TUB can tether to the inner plasma membrane via a phosphatidylinositol 4, 5-bisphospate (PIP_2) binding domain and translocate primarily into the nucleus upon G-alpha q signaling. As a result, TUB was postulated to be a signal responsive transcription factor; however, this proposed TUB-dependent transcriptional program has remained elusive.

Many of the systems impacted by loss of Tub in mammalian and non-mammalian models are reliant on cilia, antenna-like organelles cells used to sense environmental signals. One of the most striking phenotypes observed in TUB and mammalian TULP3 mutants is the mislocalization of G-coupled protein receptors (GPCRs) to cilia. Indeed, the phenotypes observed in these model systems resemble ciliopathies. How or whether TUB's nucleic acid binding properties are connected to its role in GPCR trafficking has been unknown.

Using affinity purification-mass spectrometry (AP-MS), we discovered that TUB interacts with RNA binding proteins; including ones known to be required for cilia formation and function. We found that TUB binds RNA *in vivo* and by performing individual nucleotide resolution crosslink and immunoprecpitate RNA sequencing (iCLIP-seq) we have identified endogenous TUB target mRNAs in the mouse retina and hypothalamus. Electrophoretic mobility shift assays confirmed TUB's capacity to directly bind target RNAs in vitro and helped define its binding preferences. Consistent with a role in RNA transport, select TUB bound mRNA and their encoded proteins were mislocalized in *Tub* deficient mice. A role for TUB in RNA transport was further supported by the ability of specific TUB-bound RNA sequences to redirect non-ciliary proteins to cilia. Thus, we have uncovered a novel TUB-dependent RNA regulatory program and roles for TUB in RNA transport.

These findings change our understanding of TUB domain protein functions and open new avenues for exploring signal-

responsive RNA localization and its role in appetite homeostasis and neuronal health.

1620S **Glyceraldehyde 3-phosphate dehydrogenase, spermatogenic (GAPDHS) functions as a link between melanoma metabolism and cell morphology** Marelize Snyman[1], Sophie Wix[1], Rachel Walsdorf[1], Lindsey West[1], Juliana Kim[1], Gregory A Hosler[2], Jennifer Gill[1] [1]UT Southwestern Medical Center, [2]Propath

GAPDHS is a spermatogenic enzyme expressed in most human melanomas and functions to limit metastasis in patient-derived xenograft (PDX) melanomas. To understand its role in natural tumor progression, we examined its expression in patient samples ranging from normal melanocytes to benign nevi to advanced melanomas. Using single-cell RNA sequencing data from skin, we determined that GAPDHS expression is actually detectable in normal melanocytes, which was previously not known due to the lack of granularity in bulk RNA-sequencing of skin. We confirmed this expression of GAPDHS in melanocytes at the protein level through immunohistochemistry of human skin samples. GAPDHS expression was maintained in early stages of melanocyte tumor progression from benign nevi to early melanoma. GAPDHS expression was partially or totally lost in more advanced melanomas and metastases consistent with a role in repressing metastasis. Spatial transcriptomics comparing GAPDHS high and low regions of patient melanoma tumors indicated that GAPDHS expression is associated with pigmentation and melanocyte differentiation programs. Phenotypically, lower GAPDHS expression was associated with the loss of melanoma nesting and the acquisition of less pigmented, spindle phenotypes. Knockdown of GAPDHS in melanoma PDX cells led to an increase in the mesenchymal and tumor invasive marker N-cadherin, indicating GAPDHS impacts cell morphology in addition to its previously known effects on metabolism. Additional studies are ongoing to determine whether this morphologic change is essential for its influence on melanoma metastasis.

1621S **Relating mouse and human phenotypes for cross-species translational discovery** Cynthia Smith, Susan Bello, Carol Bult The Jackson Laboratory

Mouse Genome Informatics (MGI) integrates mouse genotype-phenotype datasets from biomedical publications and large-scale projects with genomic, mutation, expression, functional and human disease model data to accelerate correlative discoveries and inform genetic disease etiology and therapeutics. To facilitate cross-species translation of phenotype and disease data between human and mouse we have developed the Human-Mouse: Disease Connection (HMDC) resource. This tool allows for users to pose simple to complex questions and retrieve phenotype and disease data for both human and mouse genes simultaneously. Human data are imported from the Human Phenotype Ontology knowledgebase. Human genes are associated with Human Phenotype ontology (HP) terms mouse genes are associated with Mammalian Phenotype ontology (MP) terms. Mappings between terms in these ontologies are included with the Mouse-Human Ontology Mapping Initiative (MHMI, https://github.com/mapping-commons/mh_mapping_initiative), which aims to collect and standardize the dissemination of mouse-human phenotype mappings and develop a set of best practices for their use. By utilizing these term mappings and relationships, users can enter a phenotype or disease term name from either species in the search, select related terms using a term picker tool, and thus return annotations using matching terms across both species in the HMDC. The results set returned includes genes associated with any of these terms or their descendants. This casts a broad net to bring in data from both species regardless of the ontology used to capture the data. The HMDC accelerates the ability to identify new candidate disease genes in human, support development of improved mouse models, and improve methods to computationally compare model characteristics to particular human diseases or syndromes.

1622S **Investigating Tuberculosis Disease Tolerance versus Susceptibility in a Diverse Murine Model** Alwyn Ecker[1], Kaley Wilburn[1], Marco Tulio Pardini Gontijo[1], Rachel Meade[2], Clare Smith[1], Martin T Ferris[3] [1]Molecular Genetics and Microbiology, Duke University, [2]University Program in Genetics and Genomics, Duke University, [3]Department of Genetics, UNC Chapel Hill

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis (TB) disease, is currently the leading cause of death by an infectious agent. Clinically, *Mtb* results in heterogenous outcomes where most individuals can contain or 'tolerate' the infection while others will go on to develop an active infection. Research has traditionally focused on identifying host and pathogen resistance pathways in the context of active TB infection. However, there is a growing appreciation for the arm of the host immune defense involved in disease tolerance. Disease tolerance manifests when host resistance mechanisms weaken, or in cases of chronic infections like TB, the host activates alternative protective mechanisms to minimize collateral damage to its tissues, while minimizing harm to the pathogen. Host and *Mtb* mechanisms that allow for individuals to 'tolerate' infection have been relatively unexplored**,** in part due to the lack of a murine model that recapitulates *Mtb*-specific disease tolerance. In published work from our laboratory, we infected over 50 Collaborative Cross mouse strains, and identified specific CC strains that recapitulate distinct TB disease states. One of these strains was CC023/GeniUNC (CC023). We have identified CC023 as a novel murine model of *Mtb*-specific disease tolerance. Upon low dose aerosol infection with ~100 *Mtb* wildtype H37Rv bacilli, CC023 maintains high bacterial burden as well as elevated levels in pro-inflammatory cytokines (i.e. *TNF-α*, IL-17, MIP-1 *α*). To elucidate host genetic factors that contribute to the disease tolerance phenotype in CC023, we have identified candidate host

genes that underlie disease tolerance. Among these genes, a top candidate gene is gasdermin A (*Gsdma*) on chromosome 11. *Gsdma* is part of the gasdermin gene family, and they are implicated in pyroptosis. The role of *Gsdma* has not been elucidated for *Mtb* infection. We infected *Gsdma*$^{-/-}$ mice to see if *Gsdma*$^{-/-}$ would phenocopy CC023's high bacterial burden and cytokine profiles, underscoring its role in CC023's disease tolerance phenotype. Our results demonstrate that *Gsdma*$^{-/-}$ mice have an intermediary *Mtb* phenotype compared to the canonical C57BL/6J (increased bacterial burden, and unique cytokine profile) but does not phenocopy the high bacterial burden and cytokine profile of CC023. These results suggest that CC023's disease tolerance is independent of *Gsdma*, and *Gsdma* may be additive rather than the driver of the disease tolerance in CC023.

1623S **Generating quantitative allelic series in mice by targeting splicing at essential exons.** Bruce A Hamilton, Dorothy Concepcion, Renee Long Cellular & Molecular Medicine, UC San Diego

Efficient modeling of genetic variation requires access to a broader palette of alleles than has historically been available in many organisms, including lab mice. Germline editing with RNA-guided enzymes has allowed construction of custom alleles for nearly a decade, but the cost of generating multiple alleles has often limited the range of alleles examined. We have targeted polypyrimidine tracts, which are essential cis-acting signals for pre-mRNA splicing, at essential exons of several developmentally important genes. Providing repair donor oligonucleotides that vary in degree of predicted splicing disruption as a pool allowed recovery of multiple alleles from each of several small-scale injection experiments. Results from editing at *Cep290* (5 alleles), *Shh* (7 alleles), *Tulp3* (6 alleles), and *Wnt1* (4 alleles) illustrate the utility of this approach to simultaneously generate both null and quantitative hypomorphic variants. Quantitative titration of gene activity allows more precise modeling of biological function, buffering and epistasis, and disease impact.

1624S **Rodent Model Resource Center (RMRC) - An international repository for novel GM rodent strains** Hsin Chih Sandy Hsu, Monica Meng-Chun Shih, Si-Tse Jiang, Hsiang-Ju Lin, Hsian-Jean Chin National Laboratory Animal Center, National Applied Research Laboratories, Taiwan

The Rodent Mouse Resource Center (RMRC) is listed as one of the repositories in the database of International Mouse Strain Resource (IMSR). RMRC is a non-profit repository under the National Laboratory Animal Center (NLAC) founded by the Taiwan government. We collected nearly 450 strains precious rodent strains generated by NLAC and domestic researchers thus far. Despite our small scale, we possess numerous globally unique strains. These unique strains are focused on disease models and tissue-specific Cre strains. These strains contribute to the advancement of research. Here are a few samples as follows,

1. Neuronal field strains
2. Metabolism field strains
3. Humanized strains

Furthermore, we provide biomaterial export/import services to distribute the strains to global researchers in need. We facilitated collaboration involving around 500 strains across more than 100 institutes. These institutes, spanning nearly 15 countries, are spread across Asia, North America, and Europe. We are trying to break through the bottleneck in Australia's exportation

We hope to assist researchers in obtaining the most suitable strains for their experiments, enabling the precious strains to reach their maximum value simultaneously. In addition, we can also accelerate the development of Taiwan's biotech and pharmaceutical industry and align with global trends.

1625S **Combining P301L and S262A tau variants prevents spheroids formation in mice** Camila A Zanella[1], Mel Feany[2] [1]Pathology, Harvard Medical School and Brigham and Women's Hospital, [2]Harvard Medical School and Brigham and Women's Hospital

Increased phosphorylation of the microtubule-binding protein tau is strongly implicated in the pathogenesis of Alzheimer's disease and related tauopathies. Tau is phosphorylated by a range of kinases at many sites. To understand the effects of serine 262 phosphorylation in vivo, we used a transgenic central nervous system (CNS) model of tauopathy. We utilized neonatal delivery of adeno-associated viruses expressing mutant tau (P301L) in non-transgenic mice. We generated full-length 0N/4R human tau constructs in the CTR4 vector, driven by a hybrid chicken β-actin promoter to ensure ubiquitous and long-lasting expression of human tau. We combined the tau variants P301L and S262A and performed behavioral analyses, including open field, novel object recognition, and fear conditioning tests. We confirmed human tau expression in the mouse brain and evaluated neuron numbers via stereology, analyzed glial fibrillary acidic protein (GFAP), and assessed spheroid formation.

Animals were aged up to 10 months. Results were first tested for normality, then analyzed using Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test or one-way ANOVA followed by post hoc Tukey's multiple comparisons. Our results show that CNS expression of P301L tau impairs long-term context memory, and preventing S262 phosphorylation shows a trend towards improving context memory. No deficits were observed in short-term memory and locomotion. Stereology did not reveal differences in the total number of neurons in the CA1 region between the tau P301L and control group. No differences were detected in GFAP levels between tau P301L and control group. Pathogenic tau is known to cause axonal degeneration, manifested as axonal swellings or spheroids, which are distinctive neuropathological features in tauopathies. In our P301L mice spheroid was observed in various brain regions, including the thalamus, cortex, and hippocampus. This was rescued when the S262A tau variant was also expressed. Preventing phosphorylation of tau at a single site was able to rescue spheroid formation. These findings enhance our understanding of how specific phosphorylation sites are implicated in tau neurotoxicity.

1626S **Genetic mapping and mediation analysis reveal immune phenotypes underlying genetic susceptibility to severe coronavirus disease in mice** Ellen L Risemberg[1], Sarah R Leist[2], Alexandra Schaefer[2], Kalika D Kamat[1], Timothy A Bell[1], Pablo Hock[1], Colton L Linnertz[1], Darla R Miller[1], Ginger D Shaw[1], Fernando Pardo Manuel de Villena[1], Ralph S Baric[1], William Valdar[1], Martin T Ferris[1] [1]Genetics, UNC Chapel Hill, [2]Epidemiology, UNC Chapel Hill

Coronaviruses have caused three severe epidemics in the 21st century, including SARS and COVID-19, while climate change and increasing human-animal interaction raise the likelihood of future outbreaks. This motivates improved understanding of viral pathogenesis and mechanisms of susceptibility to severe disease. Studies utilizing the substantial number of COVID-19 patients worldwide have identified genomic regions associated with disease severity in humans; however, the specific genes and mechanisms underlying these associations are unclear. To identify disease-associated loci and study underlying mechanisms in more depth, we created a genetic mapping population from an F2 cross between coronavirus-susceptible and coronavirus-resistant Collaborative Cross mouse strains (CC006/TauUnc and CC044/UncJ, respectively). Over 1000 F2 mice were infected with mouse-adapted SARS-CoV, SARS-CoV-2, HKU3-CoV or saline. Weight loss, lung congestion score, viral titer, and immune profiles were measured. We recently reported several loci associated with disease outcome (i.e., weight loss and congestion score) following infection with all three coronaviruses, one of which is homologous with a COVID-19-associated locus in humans. Those initial results suggested that some mechanisms of susceptibility are 1) conserved between mice and humans and 2) shared across multiple coronaviruses, including one (HKU3-CoV) not present in humans. Here, we extend that study to dissect specific mechanisms of genetic susceptibility. We perform an integrative analysis of disease related phenotypes and intermediate immune phenotypes (i.e., viral titer and immune cell concentrations) in both control and infection groups. We apply gene-by-treatment and multi-trait quantitative trait loci (QTL) mapping to identify several loci associated with immune composition at baseline (in control mice) and following infection with mouse-adapted SARS-CoV and SARS-CoV-2. We use mediation analysis to identify causal relationships underlying associated loci, which suggests that infiltration of certain immune cells and failure to control viral replication are mediators of genetically-driven disease risk. This work takes advantage of invasive phenotyping and environmental control not possible in humans to improve our understanding of coronavirus disease susceptibility, which can improve our ability to treat and control current and future outbreaks.

1627S **Characterizing Dominant Noncoding Suppressor Variants of Lethal Thrombosis in the Mouse** Arina Rodionova[1], Marisa A Brake[2], Amy E Siebert[3], Randal J Westrick[4] [1]Biological Sciences, Oakland University, [2]Harvard University, [3]Blood Research Institute at Versiti, [4]Oakland University

Thrombosis is a complex trait characterized by excessive blood coagulation in the circulatory system. Alterations in gene expression can play a prominent role in the modulation of complex traits. In C57BL/6J mice, reducing TFPI expression by 50% *Tfpi*^{tm1Gjb}*/+ akaTfpi*$^{+/-}$*)* results in a perinatal lethal phenotype when on the Factor V Leiden homozygous (*F5*^{tm2Dg}$^{L/L}$ aka *F5*$^{L/L}$) prothrombotic background. We conducted a dominant sensitized whole genome ENU mutagenesis screen to suppress this *F5*$^{L/L}$ *Tfpi*$^{+/-}$ lethality, creating large, multigenerational lines with high penetrance of *F5*$^{L/L}$ *Tfpi*$^{+/-}$ mice. We named these lines Modifier of Factor 5 Leiden (*MF5L*). *MF5L6* is a line with 85 *F5*$^{L/L}$ *Tfpi* $^{+/-}$ offspring exhibiting 72% penetrance. We performed linkage analysis and identified a significant linkage peak on Chromosome 3 (LOD=4.35) in this line, accounting for half of the suppressing effect and containing the tissue factor gene (*F3*). Tissue factor is the main protein initiator of thrombosis. In independent mouse genetic experiments, we showed that a 50% reduction in *F3* gene expression suppresses F5$^{L/L}$ Tfpi$^{+/-}$ lethality, so this became our main candidate gene in this region. To investigate the *F3* candidate in detail, we first performed DNA sequencing on the entire *F3* coding region and found no *F3* coding mutations in *MF5L6* pedigree members. We next hypothesized that a regulatory mutation was responsible for reducing *F3* gene expression in *MF5L6*. To quantify *F3* expression, I performed quantitative PCR on liver, lung, and heart tissues from *MF5L6*. I observed two distinct expression profiles in the lung, vessel, and liver *F3* mRNA of the MF5L6 mice when compared to the wild type (B6) and F3$^{+/-}$, those that had a 50% reduction in F3 expression and those who did not. Interestingly, there was no reduction of expression in the heart tissues of MF5L6, suggesting a regulatory mutation that operates in a tissue specific manner. We performed whole

genome sequencing on 5 MF5L6 pedigree members and identified two prime candidate variants in the 200 kilobase upstream region of *F3*. The mutations are not in known regulatory elements. I am presently analyzing the rest of the MF5L6 line to determine whether these mutations control *F3* expression. Identification of the variant controlling *F3* expression will provide new insights into the regulation of this important coagulation gene and will enable us to identify the variant(s) responsible for the remainder of the thrombosis suppressing effect in MF5L6.

1628S **Identification of a major *Actr2* thrombosis suppressor mutation via a sensitized ENU mutagenesis screen** Adrianna M Jurek[1], Caitlin Schneider[2], Marisa Brake[3], Amy Siebert[4], Linzi Hobbs-Parker[5], Randal Westrick[6] [1]Biology, Oakland University, [2]Michigan State University, [3]Beth Israel Deaconess Medical Center, [4]Versiti Blood Research Institute, [5]Medical Collage of Wisconsin, [6]Oakland University

Thrombosis is a complex genetic trait that occurs when blood clots form inappropriately in the veins. Using a sensitized ENU mutagenesis screen to identify novel thrombosis modifier genes, we identified a mutation in the gene *Actr2* (p. R258G, *Actr2*^{MF5L12}) that restores survival to a perinatal lethal thrombotic phenotype. *Actr2* codes for the protein ARP2, which plays a critical role in actin branching and polymerization. Previous studies showed that while *Actr2+/G* mice did not display any health consequences, *Actr2* homozygous progeny deviated from Mendelian frequencies (12% *Actr2*$^{G/G}$, p<9x10^{-7}) and developed kyphosis around 6 months of age. However, the role of *Actr2*/ARP2 in thrombosis was unknown. We hypothesized that the *Actr2*^{G} mutation suppresses thrombosis via alterations in plasma coagulation factors and/or actin cytoskeletal rearrangement. We performed biochemical and functional thrombosis assays on *Actr2+/G* and *Actr2+/+* mice to test our hypothesis. We analyzed plasma for the prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin generation (TGA) to assess blood coagulation. Platelet aggregometry was used to measure platelet activation. A mouse tail clip assay was used to measure bleeding time and quantify blood loss. To correlate these findings with actin cytoskeletal defects, we analyzed mouse embryonic fibroblasts (MEF). Our results showed that *Actr2+/G* mice had a prolonged PT and aPTT (N>17, p<0.03) and a platelet aggregation defect when compared to their wild-type littermates. There was also a significant reduction in thrombin peak height in heterozygous *Actr2* mice compared to wildtype (N=10; p=0.03). Despite the delayed time to clot formation, *Actr2*+/G mice did not exhibit a bleeding phenotype and showed no significant differences in blood loss from the tail clip assay. The MEF assay showed significant differences in mean cell surface area at 20 minutes after plating, indicating an actin cytoskeletal defect (p<0.05). Our results demonstrate extreme phenotypes in the *Actr2*$^{G/G}$ mice as a result of the profound defects in maintaining the actin cytoskeleton. However, mild coagulation pathway and platelet defects in *Actr2*+/G mice due to impaired cytoskeletal function suggest that ARP2 mediated mechanisms could be antithrombotic drug therapeutic targets that have limited effects on bleeding.

1629S **Identifying Candidate Genes for Neurodegenerative Disorders with Evolutionarily-Informed Generative Models of Protein Sequences** Evan M Cofer, Rose Orenbuch, Dinko Franceschi, Debora Marks Systems Biology, Harvard Medical School

Each individual possesses a plethora of mutations, but distinguishing pathogenic from benign mutations is non-trivial. Missense variants in particular pose a significant challenge, and their interpretation more difficult than variants that totally compromise protein function (e.g. protein truncating variants). This is particularly challenging in rare variant analyses, where we cannot simply rely on allele frequency or other proxy measures of deleteriousness. In this work, we describe the application of popEVE - an evolutionarily-informed generative model of protein sequences that leverages population data to calibrate pathogenicity predictions proteome-wide - to the task of rare variant association studies in large-scale clinical biobanks. In particular, we focus on neurodegenerative diseases such as Alzheimer's Disease, which affect millions of individuals worldwide, and are known to have a significant heritable component. In doing so, we are able to identify numerous genes relevant to neurodegenerative disorders, including many which have not been previously identified through large-scale genome-wide association studies. Many of the top candidates from our study show localized brain-specific expression, or interact with genes known to play an outsized role in the genetics of Alzheimer's Disease. Finally, we investigate specific rare variants that appear to show strong associations, and speculate about how their predicted structural changes may lead to functional changes in individual genes.

1630S **Genotype specific microbiome divergence of humanized APOE 2, 3, and 4 mice after microbiome standardization** Michelle Aries[1], Makayla Cook[1], Hope Gasvoda[1,2], Ashley Gooman[1,2], Tiffany Hensley-McBain[1,3] [1]McLaughlin Research Institute, [2]University of Providence, [3]Touro College of Osteopathic Medicine Montana

Alzheimer's disease (AD) is the leading cause of dementia in the United States. Of the more than 6.5 million people currently living with AD, almost two-thirds are female. Most cases of AD are late onset/sporadic AD (LOAD). Having the apolipoprotein E 4 (APOE4) allele is the greatest known genetic risk factor for LOAD, while the greatest known risk factor is age. The gut microbiome (GMB) is a key essential to health, and bacterial dysbiosis can lead to poorer outcomes for disease states and an increase in microbiota and their metabolites in the peripheral. The intersection of APOE, inflammation, and gut microbiota in

AD is incompletely understood. Previous studies in humans and humanized APOE mice have demonstrated *APOE*-genotype differences in commensal short chain fatty acid producing bacteria. However, these studies were cross-sectional and used methods unable to resolve the bacteria to the genus or species level. It remains unknown if *APOE* genotype is a driver of GMB divergence over time and how GMB changes with age and sex in the context of APOE genotype. In this study, humanized targeted replacement mice with either *APOE 2, 3,* or *4* (APOE-TR) were purchased from JAX. Each APOE-TR male was bred with one of the same two C57BL/6 sisters to standardize microbiomes across lines and monitor diverge based on APOE allele inheritance. Stool samples were collected from the parents at breeder set up and 6 months old, and from heterozygous pups (n=3 per sex) at wean, 7 weeks old, and 6 months old. Stool was also collected from the homozygous F2 generation (n=3 per sex) at wean and 7 weeks old. Stool pellets were assessed via shallow shotgun sequencing to increase the sequencing depth and enable species and strain level taxonomic resolution. The heterozygous pup's microbiome resembled that of their C57BL/6 mother rather than their APOE-TR fathers at wean and 7 weeks old. However, by 7 weeks old, GMB diverged based on sex and APOE genotype, with genotype-specific alterations including changes bifidobacteriaceae and lachnospiraceae. These are the first data demonstrating GMB divergence over time driven by APOE genotype. Future studies will investigate how APOE-specific bacterial species impact inflammatory responses *ex vivo* and *in vivo*. Studying genotype specific changes in GMB and how specific APOE-specific bacteria impact inflammatory responses can aid in understanding and potentially treating some of the underlining mechanisms associated with APOE-related LOAD.

1631S **The Canadian Rare Diseases Models and Mechanisms (RDMM) Network: Connecting novel disease gene discoveries to functional characterization research in model organisms** Philip Hieter[1], Sanja Rogic[1], Paul Pavlidis[1], Phillippe Campeau[2], Kym Boycott[3] [1]Michael Smith Laboratories, University of British Columbia, [2]Department of Pediatrics, University of Montreal, [3]Department of Genetics, University of Ottawa

Advances in genomics has transformed our ability to identify the molecular cause of rare diseases (RDs). Yet for most candidate RD genes, we lack insight into their biological function, how mutations identified in patients affect them, or what therapies could be useful. Model organisms (MOs) represent powerful tools to confirm the pathogenicity of RD gene variants, characterize gene's biological function, and identify potential therapies. For these reasons, the Canadian RDMM Network (http://www.rare-diseases-catalyst-network.ca/) was established in 2014 to catalyze and fund connections between clinicians discovering new disease genes and researchers able to study equivalent genes and pathways in MOs.

The RDMM Network has created a rapid and direct pathway from gene discovery to functional characterization studies in MOs. The central resource of the RDMM Network is a web-based Canadian directory of MO researchers ('the Registry') built to facilitate identifications of suitable collaborators for applying clinicians. As of Oct 2023, 694 MO researchers have registered 16,675 genes of interest. With the aid of the computational inference built into the Registry, this translates to the coverage of 9,802 human genes. RDMM uses a committee process to identify and review potential clinician-MO researcher matches and approve $30,000 CAD in catalyst funding. Since 2014, we have made 120 clinician-MO scientist connections and funded 150 functional characterization proposals. Besides the scientific insights into the molecular mechanisms of rare disease and possible novel therapies, these collaborations also lead to high impact papers, long-term collaborations, external grants.

In 2019, we established international linkages with emerging similar networks in Europe, Australia, and Japan. To facilitate community uptake, we made the RDMM Registry portable, customizable and linkable with other instances, and our committee structures and process freely available. We are willing to assist additional RDMM regional networks as needed, and to further support global collaborations. (https://rdminternational.org/)

In our third round of funding (2022-2027), RDMM expanded the scope of model systems to include the use of human cell models, including primary patient-derived cells, cell lines derived from iPSCs, and organoids. The Canadian RDMM Network will continue to create meaningful collaborations between clinicians and MO researchers and advance RD research locally and globally.

Boycott KM, et al. (2020). The Canadian Rare Diseases Models and Mechanisms (RDMM) Network: Connecting Understudied Genes to Model Organisms. Am J Hum Genet. 106(2):143-152

1632S **Multi-drug Cancer Adaptive Therapy with Deep Reinforcement Learning** Zhaozhi Li[1], Jenna Fields[2], Mohamed Osman[3], Bishoy M Faltas[3,4,5,6], Jaehee Kim[1] [1]Department of Computational Biology, Cornell University, [2]Department of Computer Science, Cornell University, [3]Division of Hematology and Medical Oncology, Department of Medicine, Weill Cornell Medicine, [4]Caryl and Israel Englander Institute for Precision Medicine, Weill Cornell Medicine, [5]Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, [6]Department of Cell and Developmental Biology, Weill Cornell Medicine

Drug resistance frequently compromises the effectiveness of standard cancer treatments, leading to tumor progression and patient relapse. Optimizing drug dosage, schedule, and sequence is crucial for delaying tumor progression and enhancing

therapeutic benefits. While previous studies on inferring optimal treatment strategies have predominantly focused on single-drug treatments, there is a pressing need to develop a computational framework based on realistic mechanistic mathematical models that can effectively address the complexities of multi-drug settings. One of the major challenges in achieving this goal is the inherent complexity and non-linearity of cancer mechanisms, further complicated by the irregularity and sparsity of clinical data. Clinicians must also adhere to established guidelines and constraints when deciding on the sequence, type, and dosage of therapy for each patient, adding an additional layer of complexity.

In this study, we developed computational frameworks with two main objectives. First, we created a personalized and scalable bio-digital twin of bladder cancer, involving a mechanistic mathematical model that captures the key biological processes driving bladder cancer progression and its response to therapy. This model was integrated into a neural ordinary differential equation, which combines the adaptability of advanced neural networks and ctDNA-derived information to provide insights into patients' genetic alterations and the impact of treatment over time. Second, we focused on inferring optimal cancer treatment strategies involving multiple drugs using deep reinforcement learning (DRL). This included the integration of clinician input to refine DRL action space; the validation of our model against clinical longitudinal ctDNA data from patients throughout their treatment; and an exploration of the interpretability of the DRL-derived treatment strategies. We applied our method to advanced bladder cancer data, demonstrating that it offers interpretable and adaptive treatment strategies for multiple drugs, thereby improving patient survival rates and overall quality of life.

Our work establishes a computational framework for investigating multi-drug cancer therapy using DRL, opening up new possibilities for personalized medicine, improving overall cancer treatment efficiency, and enhancing patient benefits. Additionally, our framework demonstrates how the use of serial ctDNA monitoring can yield valuable insights into drug responses and tumor progression in patients with advanced cancer.

1633S **A cancer variant functionalization platform using genetic interaction mapping in Saccharomyces Cerevisiae** Seevasant Indran[1], Christopher Loewen[2] [1]University of British Columbia, [2]University of british Columbia

A majority of missense variants have no functional interpretations. The remaining accounts of mutations are yet to be discovered and cannot be interpreted using traditional genetics approach. In an era where whole genome sequencing cost is approaching towards $500, this bottleneck limits the diagnostic and usability of sequencing technology. This study aims to develop and improve on a cancer variant functionalization platform using genetic interaction mapping in *Saccharomyces Cerevisiae* by an approach called Sentinel Interaction Mapping (SIM). SIM does not rely on complementation, "humanization of yeast" or human orthologs in yeast, therefore the practical limits of screening human disease associated genes is not limited to these aspects - in contrast to current orthology yeast-based screens. SIM is accomplished by transforming ~ 5000 yeast mutants with the Gene of interest (TP53) using Synthetic Gene Array (SGA) technology which identifies Synthetic Dosage Lethal (SDL) interaction partners. The associated partners termed "Sentinels", are then subjected to fitness-based assay in conjunction with the GOI potentially exposing the functionality of a variant or VUS. Functional variants under the influence of these sentinels report with a synthetic sick fitness phenotype and debilitating variants report with a fitness viable phenotype comparable to Wild Type (WT) Sentinels without the GOI. Here, we report an assay for variant functionalization of TP53 in yeast.

1634S **Quorum Sensing Underlies Viability Resurgence in Chronologically Aged Yeast Cells** Kai-Ching Hsiao, Hsin-Ying Lin, Min-Hao Kuo Biochemistry and molecular biology, Michigan state university

With the continual increase of the elderly population in modern societies, it is crucial to investigate the molecular mechanisms underlying aging in order to maximize the healthspan of the population. Using the budding yeast as the model to study the control of chronological aging, we observed the phenomenon of viability resurgence in quiescent time (VRQT), in which chronologically aged cells divide synchronously in spent medium, resulting in the extension of the population lifespan. VRQT does not require fresh nutrients. Practically all strains that we tested, including haploid and diploids, laboratory and wild strains, show VRQT readily. Progeny cells arising from VRQT repeat this phenomenon when they are old, and do not exhibit growth advantage when put in direct competition with cells that have not gone through this process. Together, these results argue strongly for an innate program that preserves population viability. Moreover, VRQT frequency is heightened by in denser cell suspension, suggesting a quorum sensing-like collective behavior. Indeed, increasing the concentration of quorum sensing molecules (QSMs) in the medium enhances the frequency of viability resurgence. On the other hand, while some cells are able to execute VRQT, there exists another population of cells that remain mitotically capable but do not engage in VRQT. These cells can be revived upon transplanting to fresh medium. Genetically, knocking *GPR1*, one of a G protein-coupled receptors in budding yeast, diminishes the occurrence of VRQT without impacting vegetative growth, suggesting that GPR1p may serve as a potential receptor for VRQT. Together, our data suggest an innate program related to quorum sensing that not only maintains the viability of aging yeast cells, but also expands a population through synchronous division through a harsh time. This

discovery may shed light strategies that enhance lifespan and healthspan.

1635S **Barcode-sequencing screen for modulators of anticancer ruthenium complex sensitivity in *S. cerevisiae*.** Andrew R Chinn[1], Jackson Blackman[2], Pamela Hanson[1] [1]Furman University, [2]UC Irvine

Phenotypes uncovered via pharmacogenomic analyses provide a viable tool for understanding the effects of diverse drugs on cellular pathways. Furthermore, these unbiased genome-wide assays can identify genes with unanticipated and/or previously unknown roles in cellular drug responses. In this study, we analyzed pools of barcoded budding yeast (*S. cerevisiae*) deletion strains to identify genes that alter sensitivity to the anticancer ruthenium complex *trans*-[tetrachlorobis(1*H*-indazole) ruthenate(III)] (KP1019). An analysis of two identical pools of barcoded yeast deletion strains was conducted using next generation sequencing, detecting a total of 3293 unique upstream barcodes representing 69.2% of nonessential deletion strains. A custom Python script was able to recover the majority of mutated barcode sequences with a Levenshtein distance of lev = 1. There was a positive correlation in the log(fold-change) of strain abundance among the two trials used in analysis (R^2 = 0.43, y = 0.61x + 0.026), indicating substantial agreement between biological replicates. GO term analysis revealed significant enrichment of DNA repair pathways amongst strains exhibiting sensitivity in both trials. This result is in line with previous studies showing DNA as a target of KP1019 in both yeast and cancer cells. Additionally, analysis of KP1019 resistant strains uncovered enrichment of the “cytosolic ribosome” GO term. As verification that the drug affects ribosomes, we monitored localization of Rpl7a-GFP. In KP1019-treated yeast, this ribosomal protein accumulates in the nucleolus indicating that the drug impairs ribosomal biogenesis. The screen also identified a number of outliers not associated with enriched GO terms. For example, yeast lacking the polyamine transporter Tpo1 consistently displayed high levels of resistance to KP1019. This result may relate to KP1019’s activation of the oxidative stress response, as published studies previously showed that *tpo1* null mutants are resistant to oxidative stress. While these results offer support for current models of KP1019 action and validate the screen design, additional characterization of novel outliers may help to elucidate the cellular pathways and processes that modulate KP1019 sensitivity. Improved understanding of the genetic factors that alter the efficacy of KP1019 may create opportunities for genetically tailored medicines and combination therapies in the future.

1636S **Suppressor screening in *S. cerevisiae DGA1*Δ *LRO1*Δ mutant reveals insights into yeast aging mechanism** Hsin-Ying Lin, Kai-Ching Hsiao, Min-Hao Kuo Michigan State University

Saccharomyces cerevisiae is a robust model organism to study aging. We reported previously that yeast cells with reduced intracellular triacylglycerol (TAG) content have a shortened chronological lifespan, whereas those with higher TAG content live much longer. Such changes are independent of the canonical function of TAG in providing energy for cells. To understand how TAG maintains lifespan, a TAG-deficient “lean” strain was subjected to high-copy suppressor screening for genes that, upon overexpression, can rescue the early death phenotype. In addition to shortened lifespan, these lean cells show elevated mitochondrial reactive oxygen species (ROS), which likely contribute, at least partly, to the lifespan shortening. Several Live-Long-Again (LiLA) candidates were identified. When cultured with the vector control, these LiLA clones acquire growth advantage when the population grow old, confirming their ability to regain normal chronological lifespan. In one of the LiLA clones that has been investigated further, reduced ROS levels and altered lipid profiles are observed, suggesting that a particular lipid species may control lifespan and metabolic metabolism when TAG is deficient. This LiLA phenotype is linked to a gene that was first described as being associated with wine fermentation in certain but not all laboratory strains. The expression, intracellular localization, and possible molecular functions of this LiLA suppressor will be discussed. In summary, our suppressor screen has unveiled novel pathways for lifespan regulation that will help our understanding and development of measures to preserve achieve healthy longevity.

1637S **Development of a High-Throughput Zebrafish Model of Blood-Brain Barrier Disruption** Sashank Sabbineni[1], Joanna R Thomas[1], William E Frye[1], Robert W Robey[1], Collin T Inglut[1], John Quinlan[1], Andrew C Warner[2], Donna Butcher[2], Jennifer Matta[2], Elijah E Edmondson[2], Alexander Y Mitrophanov[3], Blake Carrington[4], Raman Sood[4], Michael M Gottesman[1] [1]Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [2]Molecular Histopathology Laboratory, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD, USA, [3]Statistical Consulting and Scientific Programming, Frederick National Laboratory for Cancer Research, National Institutes of Health, Frederick, MD, USA, [4]Zebrafish Core, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

The blood-brain barrier (BBB) prevents entry of most drugs, including chemotherapeutics, into the brain due to a physical barrier and a chemical barrier. A physical barrier is formed due to tight junctions between capillary endothelial cells and a restrictive chemical barrier is primarily comprised of two major ATP-dependent efflux pumps (ABCG2 and ABCB1), which are expressed on the apical surface of brain microvascular endothelial cells, and function to efflux substrate drugs into the blood, preventing brain penetration. Mechanisms to disrupt these barriers are an effective way of delivering chemotherapeutics and

other drugs to the brain. However, studying these mechanisms in an *in vitro* model is challenging, and mammalian models are low throughput. On the other hand, a zebrafish model has the potential for high throughput studies and has a BBB very similar to humans. We created a zebrafish model of BBB disruption, by expressing the luciferase NanoLuc under the control of the glial-controlled promoter GFAP (glial fibrillary acidic protein) in the brain parenchyma, behind the BBB. Furimazine, a NanoLuc substrate, is unable to pass through the BBB as it is a substrate of the multi-drug efflux transporters human ABCG2 and zebrafish Abcg2a which prevent substrates from entering the brain. By introducing ABCG2 inhibitors, furimazine is able to cross the BBB and interact with NanoLuc to produce a dose-dependent increase in bioluminescence light. Younger larvae (3-5 days old) have higher baseline bioluminescence, indicating a less restrictive developing BBB whereas older larvae (7 days old) have a more restrictive developing BBB. Our future goal is to adapt this assay for high-throughput screening of BBB disruptors, and eventually combine these methods with brain tumor xenografts to assess the delivery of chemotherapeutics to brain tumors.

1638V **Exploring the impact of *S. mutans* and *L. casei* on cell cycle disruptions: insights from *C. elegans* as a model for microbial dysbiosis** Ana M. Cedeno Escobar, Katherine M. Walstrom Div. Natural Sciences, New College of Florida

Mutations disrupting the cell cycle and replication can lead to a cell becoming cancerous. Microbial dysbiosis is the term used to refer to the action of microorganisms interacting with cancerous cells to aid tumor progression and metastasis. These pathogens use their virulence factors to colonize and damage the host's cells. Specifically, the cariogenic bacterium *Streptococcus mutans* utilizes its adherence and acid-tolerant abilities to partner with other bacteria in invading the host's cells. *S. mutans* has been shown to aid the progression of oral squamous cell carcinoma and other extra-oral diseases. On the other hand, the bacterium *Lacticaseibacillus casei* exhibits probiotic properties that have been shown to aid in the treatment and prevention of intestinal cancers. As opposed to *S. mutans*, which generate microbial dysbiosis, *L. casei* could be said to generate microbial homeostasis. When grown together, these bacteria exhibit a competitive relationship where *L. casei* prevents the dominance of *S. mutans*. The intricacies of the relationship between these bacteria has not been fully elucidated. A knowledge gap remains on the effect of each species on cellular mutations having to do with cell cycle disruptions and the molecular pathways via which they act. In the present study, the effects of *S. mutans* and *L. casei* on the nematode *Caenorhabditis elegans* were explored. Two life stage assays were carried out to quantitate the amount of worms in each larval stage. One assay compared the development of worms on *S. mutans vs. L. casei*, using OP50 as a control; while the second looked at whether *L. casei* had a protective effect on the worms. Furthermore, DIC microscopy was used to image *C. elegans* hermaphrodites and eggs. A reduction in the number of offspring produced and a delay in the development of eggs and offspring were observed for worms grown on *S. mutans*. Worm growth on *L. casei* preceding transfer to *S. mutans* did not have a protective effect as evidenced by similar developmental delays and reduced egg and offspring counts. Leveraging the wealth of available information about *C. elegans* enables the correlation of observed alterations with documented changes in the literature. The hope is to introduce *C. elegans* as a model organism for oral cancer and microbial dysbiosis research, and for this study to be a stepping stone for the discovery of the molecules causing cellular aberrations.

1639V **Antizyme Inhibitor (AZIN) displays a nuclear localization in human keratinocytes (HaCaTs)** Shannon Nowotarski Penn State Berks

The polyamines putrescine, spermidine and spermine are small, naturally occurring polycationic molecules that are essential for normal cellular growth and development in eukaryotes. To generate cellular polyamines, the rate limiting metabolic enzyme ornithine decarboxylase (ODC) converts the amino acid ornithine into the diamine putrescine which is subsequently converted to the higher polyamines spermidine and spermine. Antizyme (AZ) is an endogenous inhibitor of ODC and binds to ODC targeting it to the 26S proteasome for degradation. Additionally, ODC is regulated by antizyme inhibitor (AZIN), a protein that closely resembles ODC but does not contain enzymatic activity. AZIN binds to AZ with higher affinity than ODC and therefore negates the effects of AZ on ODC. Very little is known about what other proteins AZIN might interact with in mammalian cells. Thus, the focus of these studies was to determine which proteins interact with AZIN in an attempt to better characterize the involvement of AZIN in the progression of non-melanoma skin cancer. BIOID studies were done and MYH9, or myosin 9, was determined to be a potential binding partner of AZIN. This was extremely interesting because it had recently been reported that an edited form of AZIN binds to myosin 9 in prostate cancer cells. AZIN had previously been thought to localize only in the cell cytoplasm. However, it has been shown in prostate and colorectal cancers that AZIN undergoes RNA editing by adenosine deaminase acting on RNA (ADAR1). The edited version of AZIN is able to translocate to the nucleus. In these studies, we investigated this phenomena in a human keratinocyte cell line (HaCaT) and show that, in our system, AZIN is mainly nuclear. These studies could provide rationale for the use of nuclear AZIN as a chemotherapeutic target because nuclear AZIN has been shown to be associated with more aggressive disease in prostate cancer.

1640V **the role of MYT1L and Piwil2 genes biomarkers in Medulloblastoma development** Mohammed Ali Alshehri Medical genetics, Najran university

Medulloblastoma is the most common childhood's malignant brain tumours which is about 15 – 20%. Mortality due to this disease is high (~40%) and successful treatment is associated with significant neurological and cognitive consequences, making new therapies desirable. Indeed, drivers identification for this devastating tumour will allow development of more precise targeted therapies.

Piwil2 gene as a stem cell maintaining factor that trigger spermatogenesis process has shown to be expressed in different tumour tissues and cell lines. Including, MB primary tumours, in mice. In the other hand, normal tissues showed no expression of piwil2. The tumorigenic effects of piwil2, owing to the activation of STAT3/BclX pathways. This study emphasized that overexpression of piwil2 leads to NIH3T3 highly proliferation and differentiation. However, genomic silencing piwil2 leads to decrease cellular proliferation and differentiation even leads to apoptosis.

1641V **The utilization of *Drosophila melanogaster* as a model organism to investigate the underlying mechanism of Septin11-related axonal sensorimotor polyneuropathy** Farzaneh Larti[1], Ayşe Candayan[1], Taha Akkülah[1], Gülay Kaya[1], Cansu Kocabaş[1], Mustafa Kaan Kozluca[1], Marcel Nos Lorens[1], Onur Can Aydeniz[1], Barış Can Mandacı[2], Esra Battaloğlu[1], Arzu Çelik[1] [1]Molecular Biology and Genetics, Boğaziçi University, [2]Molecular Biology and Genetics Department, Boğaziçi University

Septins are GTPases that make up the fourth component of skeletal proteins. They are found in many eukaryotes and can form a filamentous network alongside microtubules, intermediate filaments, and actin. In *Drosophila melanogaster*, Sep2 and Sep5 are the closest orthologues to Septin11. We analyzed the expression of Sep2 by using a line that expresses Sep2-GFP under the control of the Sep2 promoter. Sep2 is expressed ubiquitously in all tissues, including neuronal cells and larval muscles. Additionally, we observed that Sep2 mutants had a shorter lifespan and decreased survival rates, while about 4% showed wing and notum abnormalities.

Our investigation revealed that the absence of Septins could cause abnormal neuromuscular junctions, mispositioning of myonuclei, and defects in larval locomotion behavior. The neuromuscular junctions (NMJs) pattern in Sep2 and Sep5 mutants showed defects in innervation and misrouting phenotypes. We observed a difference in the number of boutons between Sep2 mutants and control flies, indicating an overgrowth phenotype in the absence of Sep2. We also observed poor development and overgrowth in NMJ boutons, represented by the number of satellite and super boutons. Flies with deletions in Sep2 and Sep5 showed distinctive abnormalities in developing pre- and post-synaptic areas, which implied severe defects in the dynamic localization of DLG protein at the junctions.

Furthermore, we investigated the position of nuclei in larval muscles and found that Sep2 and Sep5 mutants had clustered nuclei rather than evenly spaced nuclei, with differences in nuclear shape and volume. Moreover, the knockdown of Sep2 and Sep5 did not cause an increase in the number of flies with fragmentation in the nerve tract along the L1 vein of the wing at the age of 20 days.

The locomotion behavior of both larvae and adult flies was studied, which showed a significant decrease in the ability of mutants. Using a modified SING assay, Sep2 mutants showed defects in Startle-Induced locomotion and negative geotaxis. The crawling assay showed that the path length in 1 minute significantly decreased in Sep2 mutants. However, inducing the expression of Sep2-GFP in larval muscles could rescue that defect. Furthermore, the two-choice sucrose taste assay showed a delayed response in mutants with both Sep2 and Sep5 deletions, compared to the immediate attraction of controls.

In conclusion, this study highlights the crucial role of Septins in neuromuscular development. Further investigations are underway to assess the expression level of all *Drosophila* Septins in knockout mutants to explore compensation mechanisms. Additionally, humanized flies expressing wild-type and a truncated form of Septin11 will be used to investigate the functional orthology of human and fly Septins. Our research improves the current understanding of the mechanisms that drive neuropathic disorders.

1642V **Tissue Specific Innate Immune Responses Impact Viral Infection** Steven Miller[1], Elisha Segrist[2], Beth Gold[3], Sara Cherry[3] [1]Microbiology, University of Pennsylvania, [2]National Institutes of Health, [3]University of Pennsylvania

All organisms have many pathways to sense and respond to pathogenic challenge. The presence and regulation of these pathways can vary within tissues and cells of the same host. The CDN-STING pathway is antiviral through control of NFkB-dependent gene expression. Viruses infect diverse tissues and we set out to determine in which cell types does STING control infection and whether there are distinct gene expression programs induced in different tissues. We uncovered differences in genes induced by CDN sensing by STING across tissues. And we found that STING is required for the antiviral activity of CDNs in distinct tissues. Moreover, we found that this is important during viral infection as STING is required in particular cell types to control systemic infection. Lastly, we found that genes induced by CDN-dependent STING activation also control infection. These results highlight tissue-specific antiviral activity that likely impacts immunity to viruses with distinct tissue tropisms.

1643V **Metabolic Reprogramming in PIK3CA^{H1047R} Mutated Endothelial Cells** Brandee Rockefeller[1], Qingfen Li[2], Kevin Pumiglia[2] [1]Biology Department, Utica University, [2]Regenerative and Cancer Cell Biology, Albany Medical College

Angiogenesis, the development of blood vessels from preexisting vasculature, involves tight coordination and regulation of endothelial cell functions including migration, proliferation, and differentiation. Activation of the PI3K signaling cascade is essential to the proper formation and function of vasculature. Errors in this development can manifest as vascular malformations, which are structurally abnormal and dysfunctional blood vessel networks that have potential to cause significant morbidity and mortality. Currently, there are no uniform treatment guidelines or targeted therapeutic options as the mechanistic basis remains unclear. Over the last several years genetic profiling data has linked mutations in PIK3CA, the catalytic subunit of PI3K, to the development of vascular malformations in humans. Our goal is to better understand endothelial cell function, and the development and maintenance of PIK3CA^{H1047R} driven vascular malformations. We hypothesize, based on the robust mTOR activation and role mTOR plays in the regulation of cellular metabolism, that these endothelial cells undergo metabolic reprogramming to meet high energy and biosynthetic demands. Using a ribo-tagged lentiviral vector we were able to isolate and quantify mRNA from bead angiogenesis assays *in vitro.* We found several metabolic genes to be differentially regulated following the expression of the "hot spot" activating mutation PIK3CA^{H1047R} suggesting that endothelial cells do undergo metabolic reprogramming.

1644T **The *C. elegans* NuRD chromatin remodeler interacts with the Fanconi Anemia pathway during germline DSB repair** Deepshikha Ananthaswamy[1], Sereen El Jamal[2], Paula M Checchi[3], Teresa W Lee[1] [1]Biological Sciences, University of Massachusetts Lowell, [2]Biology, Marist College, [3]Sanford Burnham Prebys Medical Discovery Institute

All cells encounter environmental hazards that damage DNA and generate toxic double stand breaks (DSBs). Therefore, organisms have evolved overlapping and redundant DSB repair pathways to protect genome integrity, particularly in germ cells. In response to DNA damaging agents like cisplatin and hydroxyurea, the Fanconi Anemia pathway helps repair DSBs. However, since genomic DNA is always packaged as chromatin, DSB repair must be coordinated with local chromatin remodeling. The Nucleosome Remodeling and Deacetylase (NuRD) complex is one complex that repositions nucleosomes, but its role in DSB repair remains unknown. In *C. elegans*, mutations in the catalytic subunit of NuRD, CHD4/LET-418, lead to defective DSB repair and sterility. *let-418* mutants are highly sensitive to cisplatin and hydroxyurea in ways that phenocopy Fanconi Anemia pathway mutants: exposure to these DNA damaging agents causes reduced fertility, more DSBs, and severe chromosome fragmentation. We tested the genetic interaction between Fanconi Anemia mutant *fcd*-2 and NuRD mutant *let-418*, by assessing mitotic and meiotic DSB number, chromosome segregation, and embryonic survival. We find that *let-418* is epistatic to *fcd-2,* indicating that it is necessary for proper germline DSB repair. Our results suggest that nucleosome remodeling mediated by NuRD regulates DNA damage repair during oogenesis, demonstrating the importance of regulating local chromatin environment for preserving genome integrity.

1645T **Characterizing the effects of WEE-1.3 depletion on spermatogenesis in *C. elegans*** Shannon Pfeiffer, Aimee Jaramillo-Lambert University of Delaware

Meiosis, the specialized type of cell division that results in the production of eggs and sperm, is a highly complex process that is tightly regulated. This ensures that these gametes are healthy and capable of producing progeny. Though similar, meiosis in the female and male germ lines are distinct and are differentially regulated. The kinase Wee1/Myt1, which is conserved throughout eukaryotes, is a key regulator of both the mitotic and meiotic cell cycles. Wee1/Myt1 inhibits the CDK1/cyclin B complex, which is required to drive entry into M-phase. Many studies of Wee1/Myt1 during meiosis have focused on oogenesis, while its role and regulation in spermatogenesis are not well understood. Our lab uses *Caenorhabditis elegans* to study meiosis. The *C. elegans* ortholog of Wee1/Myt1 is *wee-1.3*. In *C. elegans* oogenesis, *wee-1.3* knockdown causes chromosome overcongression and premature oocyte maturation. These defects in oogenesis cause infertility, as the oocytes are unable to be fertilized by healthy sperm. In the context of spermatogenesis, *wee-1.3* knockdown had not been assessed due to experimental limitations. However, several gain-of-function (gof) mutations in *wee-1.3* are known to cause primary spermatocyte arrest, while oogenesis is unaffected. The distinct phenotypes of *wee-1.3* mutants suggest that WEE-1.3 is differentially regulated in oogenesis vs. spermatogenesis. Here, we use auxin-inducible degradation to selectively deplete WEE-1.3 from the *C. elegans* male germline. This results in severe chromosome segregation defects, including chromatin bridges and small DNA fragments, during spermatogenesis. These defects negatively impact male fertility; WEE-1.3 depleted males mated with females produce reduced brood sizes with decreased embryonic viability compared to wild type. Future directions include live imaging of spermatogenesis in WEE-1.3 depleted males, which will allow us to determine how these chromosome segregation defects arise. Since WEE-1.3 inhibits CDK-1, future work also aims to examine whether an acute loss of CDK-1 function can ameliorate the segregation defects caused by WEE-1.3 depletion.

1646T **Unveiling the function of a putative IHO-1 homolog in *Caenorhabditis elegans*** Zachary Leydig[1], Marilina Raices[1],

George Chung[2], Judith Yanowitz[1] [1]OBGYN/Reproductive Sciences, MWRI & University of Pittsburgh, [2]Biology, New York University

Meiotically dividing cells rely on the formation of DNA double-strand breaks (DSBs) to initiate crossing over. While this not only ensures genetic diversity by physically shuffling maternal and paternal genes, it also allows chromosomes to segregate appropriately by creating chiasmata between homologs. Spo11 is the enzyme that catalyzes meiotic DSBs and is highly conserved across organisms. However, Spo11 requires the activity of additional proteins to ensure it acts at the right time and in the right place. In yeast, REC114 and MEI4 were found to be essential for DSB formation. In mice, REC114, MEI4, and IHO1 form a pre-DSB complex. However, the degree to which these auxiliary proteins are conserved across multiple organisms is unknown. While homologs have been found for REC114 and MEI4 in the worm (DSB-1/2 and DSB-3, respectively), an ortholog for IHO1 remains elusive. We recently identified a putative homolog of IHO-1 in the worm using degenerative amino acid alignments. We removed the coding sequence with CRISPR to create a null allele. Unlike DSB gene mutations which confer a HIM phenotype and show univalents in diakinesis oocytes, *iho-1* mutants show DNA fragments and fusions, consistent with a repair defect and not a DSB defect. Yeast two-hybrid further revealed no interactions with worm DSB proteins supporting the conclusion that the worm homolog is functionally divergent. We will present these results and further studies on the function of this protein in the worm germ line.

1647T **Characterization of the double-strand break machinery in C. elegans** Marilina Raices[1,2], Fabiola Balmir[3], Wei Li[3,4], Nicola Silva[5], Judith Yanowitz[2,6] [1]Ob/GYN, Magee-Womens Research Institute, Pittsburgh, [2]University of Pittsburgh, [3]Magee-Womens Research Institute, [4]Tsinghua U. Medical School, [5]Masaryk University, [6]OB/GYN, Magee-Womens Research Institute

Crossing over between homologous chromosomes during meiosis promotes genetic diversity by creating new combinations of alleles over generations. Crossovers (CO) create physical connections between the homologs that promote their proper alignment on the meiotic spindle and subsequent apposite segregation. Defects in CO formation can trigger errors in the correct number of chromosomes in the gametes. Recombination initiates with a double-strand break (DSB) introduced by the conserved topoisomerase-like enzyme SPO-11 but the timing, placement and number of COs depend on the activity of accessory factors. In fact, SPO-11 works in concert with at least 10 other proteins to ensure the proper timing and placement of breaks (and COs). While these proteins have been well characterized in budding yeast, our understanding of the conserved complexes that regulate SPO11 are still poorly understood.

In C. elegans, dsb-1, him-17, mre-11, and rad-50 are required for DSBs formation. xnd-1 and him-5 influence DSB formation mainly on the X chromosome. dsb-2 impacts breaks on all chromosomes and becomes essential in older animals. rec-1 influences CO placement and cep-1, lin-35, and parg-1 contribute to break induction. We are investigating the relationship between these DSB factors in order to uncover the regulatory events that promote DSB formation. To this end, we are taking a multi-pronged approach using genetic epistasis with partial loss-of-function alleles, rescue with ectopically expressed transgenes, yeast-2-hybrid analyses, and co-immunoprecipitation. These studies are allowing us to construct a model for the timely recruitment and activation of SPO-11 that is revealing remarkable conservation across evolution.

1648T **HP1 recruits the chromosomal passenger complex to the chromosome for acentrosomal spindle assembly in meiosis** Siwen Wu[1], Manisha Persaud[1], Keara Greer[1], Kim McKim[2] [1]Rutgers University, [2]Genetics, Rutgers University

Chromosome segregation fidelity during female meiosis is critical for maintaining genome integrity, with aberrations causing infertility, miscarriages, and severe congenital anomalies. At the heart of this process, the chromosomal passenger complex (CPC), comprising the inner centromere protein (INCENP), Borealin, Survivin, and Aurora B kinase, functions as a central regulator of spindle assembly and ensures accurate bi-oriental chromosome segregation during meiotic cell division. In Drosophila oocytes, the CPC is required for microtubules recruitment to chromosomes, facilitating acentrosomal spindle formation post-nuclear envelope breakdown and managing kinetochore-microtubule attachments during meiotic metaphase I. However, the mechanics behind CPC's recruitment to the chromosomes and its interaction with spindle microtubules remain elusive. INCENP is a scaffolding protein providing the platform for CPC assembly. It encompasses several essential domains, including a N-terminal domain responsible for binding Borealin and Survivin, the STD and SAH domains known to interact with microtubules (MTs), and a heterochromatin protein-1 (HP1) binding domain. From previous study, we hypothesized that HP1 recruits the CPC to the chromosomes to initiate acentrosomal spindle assembly. To test this hypothesis, we developed HP1 RNAi reagents and generated INCENP mutants with deletion of HP1 binding sites. In HP1 RNAi oocytes treated with Binuclein 2 (BN2), an Aurora B inhibitor, we found no CPC recruitment to chromosomes. This observation indicated that HP1 is required for CPC's chromosomal recruitment. Interestingly, while INCENP mutants deleting the HP1 binding regions exhibited spindle formation, many HP1 RNAi oocytes did not, suggesting that HP1 is required for spindle assembly but its interaction with INCENP isn't essential for this function. Both HP1 RNAi and INCENP mutants displayed chromosome biorientation defects, indicating that HP1-INCENP interaction is critical for chromosome biorientation. Although bipolar spindle is being formed,

there must be defects in how the kinetochore and microtubules interact. Because a HP1-INCENP interaction is not required for spindle formation, a more complicated regulatory system for spindle assembly, possibly involving multiple INCENP domains or Borealin, may be required. Together, our findings shed light on the intricate dynamics of CPC-HP1, enriching our understanding of bipolar spindle organization and meiotic chromosome segregation in female meiosis.

1649T **Effects of B Chromosomal Dosage on Longevity Outcomes in *Drosophila melanogaster*** Tristan E Young[1], Rachel Ferris[2], Nicole C Riddle[1] [1]Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

Sex-specific longevity is poorly understood across the animal kingdom, with some species having one sex live longer than the other and some species having no sex-based differences in longevity. In humans and many other mammal species, females tend to live longer. This trend is also seen in many strains of *D. melanogaster.* There is, however, no known reason for this variation in sex-based aging differences. One molecular pathway of interest in aging research is heterochromatin maintenance. The heterochromatin loss model of aging was first proposed in 1997 by Villeponteau. This model suggests that chromatin maintenance is lost with age, leading to genomic instability, negatively impacting lifespan. Furthermore, this model suggests that altering the amount of heterochromatin might impact lifespan. One way to test heterochromatin's roles in aging and longevity is by exploiting non-essential B chromosomes, which are small chromosomes comprised primarily of large amounts of heterochromatin. A small percentage of eukaryotes, such as *D. melanogaster,* can carry B chromosomes, making them an ideal model organism for testing heterochromatin dosage roles in longevity. We utilized *Drosophila* strains with and without B chromosomes to perform a longevity study. Preliminary data suggest a positive association between B chromosome dosage and longevity in both sexes. On-going experiments investigate measures of genome stability in these strains to determine if differences in heterochromatin maintenance might explain the lifespan increase seen in the B chromosome strains. Investigating heterochromatin dosage effects in *D. melanogaster* will inform the roles of heterochromatin in aging and lifespan across animal species.

1650T **The Role of Drosophila Rif1 and CtIP in DNA Double-Strand Break Repair** Makenzie S Thomas, Gautham Pillai, Margaret Butler Human Science, Georgetown University

Double-strand breaks (DSBs) are genotoxic DNA lesions that must be accurately and efficiently repaired to maintain genome integrity. DSBs are largely repaired through non-homologous end-joining (NHEJ), homologous recombination (HR), or single-strand annealing (SSA). Rif1 and CtIP are conserved proteins implicated in DSB repair pathway choice through their potential redundant roles in DNA end resection required for homology-directed repair. To elucidate the role of Rif1 and CtIP in error-free HR repair, the DSB reporter assay, Direct Repeat of white (DR-white) was performed in Drosophila melanogaster. Our work demonstrates a ~20% decrease in HR in DmCtIPΔ mutants and ~7% decrease in HR in DmRif1Δ mutants compared to heterozygous controls. Similar to DmCtIPΔ mutants, DmRif1ΔCtIPΔ double mutants experienced a ~20% decrease in HR compared to heterozygous controls. Tracking across Indels by DEcomposition (TIDE) molecular analysis was utilized to assess the role of DmRif1 and DmCtIP in DSB repair pathway choice via NHEJ. These results revealed a ~30% increase in NHEJ for DmCtIPΔ mutants (with a proportional decrease in HR) and no difference in NHEJ for DmRif1Δ mutants. Once again, compared to the DmCtIPΔ mutants, DmRif1ΔCtIPΔ double mutants experienced a similar increase in NHEJ of ~30% (with a proportional decrease in HR). Interestingly, while we show a ~30% decrease in SSA for DmCtIPΔ mutants using the P{wIw} SSA assay, there was no difference in SSA repair for DmRif1Δ mutants, which may suggest a more extensive end resection function for CtIP. Future directions will include SSA analysis of DmRif1ΔDmCtIPΔ double mutants to elucidate potential redundant roles of Rif1 and CtIP in extensive end resection, ultimately impacting DSB repair pathway choice.

1651T **Fanconi Anemia Protein FANCD2 Promotes Homologous Recombination Repair in Drosophila** Caroline Worrell, Sara Martin, Anna Joseph, Mitch McVey Tufts University

The Fanconi Anemia pathway is made up a group of proteins that work together to repair interstrand crosslinks (ICLs) and protect stalled replication forks. Mutation of any of the Fanconi Anemia (FANC) genes results in Fanconi Anemia, a harmful human disease that impacts bone marrow development and causes predisposition to various types of cancer. The FANC proteins have also been shown to interact with other types of DNA damage repair processes, and recent published research has shown interaction between FANCD2 and DNA polymerase theta. Polymerase theta is the main protein involved in alternative end joining, an error-prone repair mechanism used to repair DNA double-stranded breaks. Given this observed relationship, the goal of this project is to investigate whether two FA proteins, FANCD2 and FANCI, also play a role in alternative end-joining repair. To test this, a transposon-based site-specific double-strand break repair assay was performed in order to observe the effects of mutations in these two proteins on the frequency of homologous recombination and alternative end-joining repair. The results of this assay showed an increase in alternative end joining and a decrease in homologous recombination repair in the *fancd2* mutants. In addition, we observed a high level of male sterility in *fanci* mutants experiencing transposon-induced breaks. Currently, we are conducting experiments to more clearly define the role of FANCD2

in homologous recombination and to characterize other independent functions of FANCI that promote genome stability in Drosophila.

1652T **An Extended D-loop or a Migrating Bubble? A DNA Gap Repair Assay Based on APOBEC-Induced Mutational Signature to Assess D-loop Dynamics** Mohamed Mahmoud[1], Jeff Sekelsky[2] [1]Department of Genetics, University of North Carolina at Chapel Hill, [2]Department of Biology, University of North Carolina at Chapel Hill

Revealing gap repair mechanisms is not only important for our understanding of genome integrity maintenance, but it is also important for optimizing applications such as Cas9 gene drive and genome editing. Different aspects of gap repair require more investigation, including strand invasion dynamics. Two models have been hypothesized to explain D-loop dynamics: an extended D-loop model and a migrating bubble model. Moreover, it has been hypothesized that gap repair involves several rounds of resection, strand invasion, and synthesis. To fill this knowledge gap, we will use the ebony gene locus as a target sequence for Cas9 cutting and we will use a template sequence on the homologous chromosome. We built different templates, representing different gap lengths. we will use APOBEC3A-mediated mutagenesis to determine where ssDNA is during the repair. Using long-read sequencing, we will analyze target and template sequences to detect APOBEC3A-induced mutations, their patterns, and what model they support. Furthermore, mutations in sequences flanking the target sequence will indicate the extent of resection. Finally, we will carry out experiments in mismatch and base excision repair mutant flies as APOBEC3A mutations leave behind genomic uracil and mismatches that can be fixed by base excision repair (BER) and mismatch repair (MMR), respectively.

1653T **New Insights into the structure and formation of the *D. melanogaster* B chromosome** Shania Kalladanthyil[1,2], Stacey L Hanlon[1,1,2] [1]University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

Comparative studies between closely related species reveal that genomes are constantly undergoing changes to their chromosome composition; however, these species can be separated by millions of years of evolution, obscuring the context of how new chromosomes form. The supernumerary, non-essential B chromosomes that arose less than 20 years ago in *Drosophila melanogaster* are a newly established model system that has the potential to provide valuable insights into how new chromosomes form and change in real time. The current model of how these B chromosomes arose is through a centromeric misdivision event on chromosome 4 that led to the fusion of the heterochromatic arm fragments to produce an isochromosome. Genomic analysis of the B chromosome centromere shows that it is from chromosome 4, however there is no structural signature that is indicative of a centromeric misdivision event. To further investigate the structure of the B chromosome, we performed fluorescent in situ hybridization (FISH) on metaphase chromosome spreads in larval neuroblast tissue using probes that bind specifically to the chromosome 4 centromeric island *Lampedusa*. Consistent with our genomic data, we found that *Lampedusa* was enriched on the B chromosomes compared to chromosome 4 within the same metaphase spread. These results lead us to propose an alternative model of B chromosome formation that is a result of a double stranded breakage event distal to the centromere to produce an isochromosome with two centromeres. To test this model, we are taking advantage of advances in ultra-long-read (ULR) sequencing technology to create a *de novo* assembly of the B chromosome. We are currently setting up a reliable workflow for extracting high molecular weight DNA from *Drosophila* embryos for ULR resequencing, followed by a pipeline to assemble these long reads into a genomic map of the B chromosome. Our goal is to use this map to confirm the presence of two centromeric islands on the B chromosome, pinpoint the location of the original breakage event, and reveal the genomic context that enabled this break to occur. The B chromosomes are an emerging model system for the empirical study of *de novo* chromosome formation, ultimately leading to a deeper understanding of how new chromosomes arise and evolve across all life.

1654T **In vivo studies of the *Drosophila melanogaster* AP endonuclease 1 ortholog Rrp1** Alyssa Persano, Sara Martin, Mitch McVey Tufts University

Critical for maintaining genomic integrity, loss of DNA repair proteins can have major implications for cellular and organism health. One such protein is the mammalian apurinic/apyrimidinic endonuclease 1 (APE1) which primarily functions in the base excision repair (BER) pathway. In response to damage often caused by reactive oxygen species, a glycosylase removes the damaged base, leaving an AP site. APE1 then primes DNA synthesis by cleaving the sugar-phosphate backbone. While APE1 has been well-characterized in BER, embryonic lethality in APE1 knockout mice has made it challenging to examine the molecular targets of APE1. Although APE1 can be embryonic lethal, the *D. Melanogaster* homolog for APE1, recombination repair protein 1 (Rrp1), has been shown to be viable through embryonic development. Preliminary experiments in *D. Melanogaster* reveal *Rrp1* null mutants have an average hatching rate around 86.78%, which is comparable to wild-type hatching rates. Given this unique viability of Rrp1 mutants and overall significance of APE1 in mammalian disease pathology, *D. Melanogaster* is a strong candidate to help elucidate crucial APE1 mechanisms. Following mammalian models, the first step is to test if *Rrp1* mutants are sensitive to DNA damaging agents that cause base damage, such as methyl

methanesulfonate. We hypothesize that *Rrp1* mutants will be deficient in BER and therefore hypersensitive to DNA damaging agents. Sensitivity assays, which measure the relative survival of *Rrp1* mutants treated with DNA damaging agents, are in progress. In addition to mutagen sensitivity, APE1 has been hypothesized to impact the intracellular redox state which is important in neurodegeneration as neurons are especially vulnerable to oxidative damage. Given the implications of APE1 in neuronal genomic integrity and the high expression of Rrp1 in the *D. Melanogaster* nervous system, we further hypothesize that *Rrp1* mutants will display neurologic phenotypes. To test this hypothesis, we will use behavioral assays and brain histology to evaluate climbing ability and brain tissue health in *Rrp1* deficient flies With these assays, the role of Rrp1 in *D. Melanogaster* BER and neurodegeneration can be more clearly understood, potentially revealing a novel avenue by which to explore crucial neurologic disease pathology and AP endonuclease activity.

1655T **Performance, Modifications and Tuning: Post-translational modifications on Corolla, a meiotic synaptonemal complex protein** Adam Bomar, Katie Billmyre Genetics, University of Georgia

Post-translational modifications (PTMs) greatly increase the diversity and functionality of proteins encoded by the genome. They can affect the shape of a protein, how it interacts with other proteins, and protein synthesis. One process that is heavily dependent on PTMs is meiosis. Meiosis is a multi-stage process during which germ cells go through two rounds of division to split into gametes (e.g. eggs and sperm) containing half the amount of genetic information. Specific proteins are needed at different stages to ensure the cell cycle is proceeding correctly, and chromosomes are separating accurately. Improper chromosome segregation can lead to a number of genetic syndromes and infertility. The lab is currently studying PTMs of a multi-protein structure, the synaptonemal complex (SC) that holds DNA together during early meiosis. The SC forms between the arms of homologous chromosomes during early meiosis and is necessary for the proper segregation of chromosomes. In mice and *C.elegans*, disruption of cyclin dependent kinase (CDKs) activity leads to a loss of functional SC. However, in Drosophila melanogaster we have very little information about SC regulation during meiosis. Here we focused on a central element protein called Corolla. On Corolla, we identified three putative CDK binding sites located near potential phosphorylation sites. To investigate these putative sites, we used CRISPR to create flies lacking them. Corolla^{3xCDK} females did not properly assemble the SC and contained polycomplexes, large aggregates of SC proteins.

Currently, we are characterizing other aspects of meiosis such as double-strand break formation and recombination in these mutants. Additionally, we are taking a two-pronged approach to determine what CDKs/cyclins are active during meiosis. First, we are examining the SC in flies where specific CDKs/cyclins have been knocked down in the germline using RNAi. Second, we are using yeast 2-hybrid to look for physical interactions between CDKs/cyclins and SC components. This work will increase our understanding of what regulates SC assembly and maintenance.

1656T **Not Always Precise: CRISPR/Cas9 Utilizes Multiple Mechanisms of Homology Directed DNA Repair Including Novel Models and Some Detrimental Outcomes** Evan Dewey, Reese Perini, Jeff Sekelsky Genetics, University of North Carolina-Chapel Hill

Accurate understanding of homology directed DNA repair (HDR) non-crossover and mitotic crossover mechanisms is critical to genetic disease prevention. Precisely regulated HDR specifically avoids crossovers to promote non-crossovers, avoiding loss of heterozygosity and genetic disease. We have the unique ability to accurately interpret HDR mechanisms by preserving single nucleotide polymorphisms to define heteroduplex DNA that is key to analysis within crossover and non-crossover products through complete mismatch repair knockout in *Drosophila*. Using this tool, we have identified mechanisms of several independent Cas9 induced mitotic crossover and non-crossover events, including some novel and detrimental outcomes. We see bias toward one pattern of double Holliday Junction (dHJ) cleavage (resolution) in mitotic crossovers, as well as crossovers not predicted by traditional dHJ resolution models, suggesting novel mitotic crossover models. We also find heteroduplex DNA resulting from synthesis is longer on the 5' side of the double strand break (DSB) site in both crossovers and non-crossovers, indicating preference for synthesis in the 5' direction over 3'. We hypothesize this 5' synthesis preference stems from persistent binding of Cas9 to the plus strand after cutting, inhibiting that strand from invading the template, favoring minus strand invasion, and thus facilitating the 5' synthesis bias. In some non-crossovers we see detrimental outcomes such as transposable elements aberrantly inserted near the DSB, large (>50 bp) deletions, and small (4-10 bp) deletions caused by DNA polymerase theta-mediated end joining. We hypothesize these stem from repeated Cas9 cutting, underscoring the importance of caution when employing CRISPR/Cas9 gene editing, as very efficient guide RNAs can cause detrimental outcomes. Our work enhances knowledge of HDR mechanisms, expanding how mitotic crossovers lead to genome instability, and providing better understanding of beneficial HDR utilization in CRISPR/Cas9 genome editing.

1657T **Genomic studies investigate how DNA replication regulates histone incorporation in the *Drosophila* male germline** Jennifer Urban[1], Daniel Ringwalt[1], John Urban[2,3], Brendon Davis[1], Wingel Xue[1], Wai Lim Ku[4], Ryan Gleason[1], Keji Zhao[4], Xin Chen[1,3] [1]Biology, Johns Hopkins University, [2]Carnegie Institution for Science, [3]Howard Hughes Medical Institute, [4]National Heart, Lung, and Blood Institute

As a multicellular organism develops, DNA replication in a mother cell duplicates the genome, followed by mitosis to generate two daughter cells with identical genetic information. How this cell division process contributes to diverse cell types is a fundamental question in developmental biology. It is well known that epigenetic mechanisms play a role in cell fate determination. For example, histones and their post-translational modifications influence chromatin structure to help define cell identity.

We hypothesize that asymmetric cell division contributes to cell diversity through differential inheritance of epigenetic information like histones. To support this, we discovered distinct inheritance patterns for histones during asymmetric cell division of *Drosophila* male germline stem cells (**GSC**s). While pre-existing histones are retained in the self-renewing stem cell, newly synthesized histones are enriched in the differentiating daughter. Further studies demonstrate that at individual DNA replication loci, old histones recycle to the leading strand while the lagging strand incorporates new histones. As leading and lagging strands switch at replication initiation and termination zones, it is unclear how local replication fork asymmetries produce sister chromatids enriched for either old or new histones.

My research tests the question, "how does DNA replication contribute to histone asymmetries that influence cell fate?" Before addressing this, I must first define the epigenetic and DNA replication landscapes in GSCs. However, genomic approaches are challenging in heterogenous tissue with low cell numbers. Using a *Drosophila* testis tumor to increase GSC number, I adapted a chromatin immunocleavage (ChIC) assay to define chromatin features for germline versus somatic gonadal cells. Information on H3K4me3-, H3K27me3-, and H3K9me2/3-enriched regions was integrated with cell type-specific RNA-seq to compare how chromatin relates to cell-specific transcriptional output. Additionally, I performed nascent DNA profiling (Repli-seq) to understand how replication progresses through different chromatin landscapes of each cell type. All three data sets are compared with each other and between cell types to address my hypothesis that a distinct replication program in GSCs contributes to their unique chromatin landscape and cell fate. Altogether, my research aims to address how DNA replication-coupled histone incorporation establishes global histone asymmetries in the *Drosophila* male germline.

1658T **Combinatorial effect of environmental heavy metals on genome stability** Tin Tin Su[1], Elle Mcdonald[2], Barbara Frederick[2] [1]University of Colorado, [2]UNIVERSITY OF COLORADO, BOULDER

Environmental heavy metal contamination poses significant health risks to communities near industrial sites, typically those with lower-income populations. Due to past and current mining activity, the state of Colorado contains several contaminated sites. One such site is in Pueblo, Colorado, where lead (Pb), arsenic (As) and cadmium (Cd) are major heavy metal contaminants in surface soil. Pb replaces essential metals in the body to inhibit the function of enzymes including those used to reduce reactive oxygen species (ROS). As binds sulfhydryl groups and can replace phosphorous, thereby impeding enzymes including those used for DNA repair. Cd has been shown to catalyze ROS production. ROS can damage DNA and lead to genome instability. Indeed, exposure to Pb, As and Cd is associated with an increased risk of various types of cancer including lung and brain cancers. Historically, studies of the genotoxic effect of heavy metals have typically focused on one metal at a time. In contaminated environments, however, these metals appear in combination. Available data show that pollutant combinations, for example Pb and Black Carbon, can synergize in ROS production and DNA damage, with the resulting effect greater than the sum of the effect of each agent. Yet, the genotoxic effects of Pb, As and Cd in combination have not been studied systematically. To fill this knowledge gap, we are assessing the effect of combinations of heavy metals on the genome in two experimental models, human epithelial cells and *Drosophila* larvae. In *Drosophila*, we are quantifying lasting genomic changes in terms of Loss of Heterozygosity (LOH), using fluorescent reporters we described in recent publications (Brown et al., 2020, PLoS Genetics, PMID: 33075096 and Brown et al., Genetics, 2023, PMID: 37214983). In human cells, we are using comet assays and antibody staining to gamma-H2Ax to monitor the level of DNA damage. Results from these studies will be presented.

1659T **Investigating the influence of the *TM3, Sb Ser* balancer chromosome on the female meiotic drive of B chromosomes in *D. melanogaster*** Ryan M Gado[1], Stacey L. Hanlon[1,2] [1]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

According to Mendel's Law of Segregation, the meiotic segregation of a genetic element should be random, resulting in a 50-50 pattern of inheritance. Selfish genetic elements, such as the supernumerary, nonessential B chromosomes, can violate Mendel's law and exhibit preferential transmission during female meiosis, a phenomenon known as meiotic drive. The B chromosomes recently discovered in *D. melanogaster* are not themselves selfish, however the genetic background they arose in enables them to exhibit selfish behavior during female meiosis and be preferentially transmitted from one generation to the next. Their progenitor stock carries a null allele of *matrimony* (*mtrm*^{126}) held over the *TM3, Sb Ser* (*TM3*) balancer, both of which are necessary but not sufficient to induce robust meiotic drive of the B chromosomes. Since it is currently unknown how *TM3* contributes to this drive, we hypothesize that one or more of the ten inversion breakpoints on *TM3* interrupt a gene that confers drive suppression, allowing for a stronger degree of drive when *TM3* is present. *TM3* carries five large

inversions that interrupt seven genes, such as the well-known tumor suppressor gene *p53*, one intergenic region, and two unknown regions. To explore our hypothesis, we used a screening assay to monitor the segregation of chromosome 4 that serves as a proxy for B chromosome segregation as both chromosomes exhibit biased transmission in a drive-permissive genetic background. Preliminary screening of the candidate drive suppressors revealed that a reduction in *p53* contributes to biased chromosome segregation, indicating it may be a suppressor of drive. To confirm this result, we are assessing the B chromosome transmission in a *p53/mtrm*^{126}mutant background to determine if *p53/mtrm*^{126} females can recapitulate the high level of drive seen in a *mtrm*^{126}*/TM3* background. Ultimately, this work will provide the starting point for uncovering the mechanism in which the *TM3* balancer chromosome enables the robust drive of the B chromosomes.

1660T **A Cytological F1 RNAi Screen for Defects in *Drosophila melanogaster* Female Meiosis** William D. Gilliland Biological Sciences, DePaul University

Genetic screens induce mutations, make the mutated chromosomes homozygous, and then assay those homozygotes for the phenotype of interest. When screening for genes required for female meiosis, the phenotype of interest has typically been nondisjunction from chromosome segregation errors. As this requires that mutant females be viable and fertile, any mutants that are lethal or sterile when homozygous cannot be recovered by this approach. To overcome these limitations, our lab has screened the VALIUM22 collection produced by the Harvard TRiP Project, which contains RNAi constructs targeting genes known to be expressed in the germline in a vector optimized for germline expression. By driving RNAi with GAL4 under control of a germline-specific promoter (*nanos* or *mat-alpha4)*, we can test genes that would be lethal if knocked down in all cells, and by examining unfertilized metaphase-arrested mature oocytes, we can identify defects associated with genes whose knockdown results in sterility or causes other errors besides nondisjunction.

We screened this collection to identify genes that disrupt either of two phenotypes when knocked down: the ability of meiotic chromosomes to congress to a single mass at the end of prometaphase, and the sequestration of Mps1-GFP to ooplasmic filaments in response to hypoxia. After screening >1450 lines of the collection, we obtained multiple hits for both phenotypes, identified novel meiotic phenotypes for genes that had been previously characterized in other processes, and identified the first phenotypes to be associated with several previously uncharacterized genes.

1661T **Comprehensive Tissue-Specific Somatic Mutation Profiling via RNA-seq in Diverse Mice** Alexis Garretson[1,2], Beth L Dumont[1,2] [1]Genetics, The Jackson Laboratory, [2]Tufts University

Somatic mutations are genetic alterations that occur in non-germ cells and are thus not passed on to offspring. However, somatic mutations can be transmitted to the progeny of mutated cells and disrupt normal cellular processes, leading to dysregulation or oncogenesis. Indeed, somatic mutational loads are associated with cancer, aging, neurodegeneration, autoimmune diseases, and many other genetic diseases. Tumor whole-genome sequencing has led to the identification of characteristic mutation signatures associated with distinct tumor types, but somatic mutation profiles have been far less extensively studied in healthy tissues. To address this gap, we leverage multi-tissue RNA-seq data from genetically diverse mouse strains to comprehensively identify somatic mutations in 10 tissues. We define tissue-specific mutation rates, spectra, and signatures that reveal associations with gene expression levels, chromatin states, and metabolic phenotypes. For example, we identify higher mutation rates in islet cells of mice maintained on a high fat diet and demonstrate that increased mutation rates in this context are associated with a mutational signature of dietary exposure to aflatoxin, a common fungal pathogen of grains. In addition, we find that voluntary exercise is positively associated with increased mutation rate in skeletal muscles and linked to a mutational signature driven by reactive oxygen species. Our findings reveal tissue-specific patterns of somatic mutations and shed light on the relationships between metabolic and molecular phenotypes in healthy tissues, prior to tumorigenesis. We are currently extending our somatic mutation calling method to a large, outbred population of mice for which we have RNA-seq data from multiple tissues. This unique resource will allow us to use QTL mapping to identify genetic loci influencing somatic mutation profiles and rates in healthy mammalian tissues for the first time.

1662T **Meiotic chromosome segregation in the holocentric pantry moth *Plodia interpunctella* can occurs through multiple mechanisms dictated by crossover position** Leah F. Rosin[1], Elissa P. Lei[2] [1]National Institutes of Health, NICHD, [2]National Institutes of Health, NIDDK

Precisely regulating chromosome dynamics during meiosis is essential for reproductive success across species. Errors in meiotic chromosome segregation can lead to reduced fertility, miscarriages, or chromosomal disorders, such as Down Syndrome or Turner Syndrome. Yet, the mechanisms underlying meiotic chromosomal events such as homolog pairing and chromosome segregation are not fully understood in most species. Furthermore, even in species where meiotic chromosome dynamics are beginning to be elucidated, whether or not mechanisms are conserved across species is unclear. Here, we employ Oligopaint DNA FISH to investigate mechanisms of meiotic homolog pairing and chromosome segregation in the

holocentric pantry moth *Plodia interpunctella* and compare our findings to our previous studies in the silkworm moth, *Bombyx mori*, which diverged from *P. interpunctella* over 100 million years ago. We find that like *Bombyx*, meiotic pairing during *Plodia* spermatogenesis is initiated at gene-rich chromosome ends. Additionally, both species form cruciform-like bivalents at metaphase I. However, unlike the telokinetic chromosome segregation mechanism observed in many holocentric species such as *Bombyx* and *Caenorhabditis elegans, Plodia* employ a novel "monokinetic" mechanism of meiotic chromosome segregation, where either telomeres or chromosome middles can act as a kinetochore-recruitment site during meiotic divisions. This monokinetic segregation is uniquely influenced by crossover position, where central kinetochore formation is likely achieved by the unusual formation of multiple chiasmata per chromosome despite the small size of *Plodia* chromosomes. Together, our data reveal conserved mechanisms of homolog recognition and pairing initiation but diverged mechanisms of chromosome segregation, and possibly crossover regulation, across Lepidopteran insects.

1663T **The role of dual histone methylation readers ZCWPW1 and ZCWPW2 in PRDM9 dependent meiotic recombination** Dawn Watkins-Chow[1], Mohamed Mahgoub[2], Sherry Ralls[1], Melania Bruno[1], Jada Gonzales[1], Meghan Yamasaki[3], Florencia Pratto[4], Todd Macfarlan[1] [1]NICHD, NIH, [2]Washington University in St. Louis, [3]NICHD Reproductive Endocrinology and Infertility Training Program, NIH, [4]NIDDK, NIH

Successful meiosis is an intricate process requiring the pairing and segregation of homologous chromosomes. Errors during meiosis can result in aneuploidy, a leading cause of pregnancy loss. Synapsis, the process that forms a physical linkage between homologous chromosomes is facilitated by the repair of DNA double-strand breaks (DSBs) that initiate homologous recombination. The location of DSBs on homologous chromosomes is determined by the DNA binding methyltransferase, *Prdm9*, one of the fastest evolving genes in the mammalian genome and the only known mammalian speciation gene. PRDM9 methylates histone H3 on lysines 4 and 36 which is essential for DSB formation and subsequent homology-directed repair, which can result in crossovers or smaller gene conversion events at PRDM9-initiated hotspots. We have identified two genes, *Zcwpw1* and *Zcwpw2,* encoding dual histone methylation readers that co-evolved with and are tightly co-expressed with PRDM9 during spermatogenesis. The two paralogous genes share conserved zf-CW and PWWP histone binding domains and both ZCWPW1 and ZCWPW2 bind dual methylated (HeK4me3 and H3K36me3) peptides. *In vivo* knockout of either *Zcwpw1* or *Zcwpw2* results in azoospermia and complete infertility, confirming the two genes are not functionally redundant during spermatogenesis, and loss of either paralog results in defective pairing of homologous chromosomes during synapsis. Characterization of the DNA double strand break landscape in *Zcwpw1* or *Zcwpw2* mutants suggests that ZCWPW2 and ZCWPW1 play distinct roles in DNA double strand break formation and repair, respectively. Work is ongoing to identify interacting proteins to further explore the mechanism of ZCWPW1 and ZCWPW2 in meiotic recombination. Collectively our data suggests that *Zcwpw1* and *Zcwpw2* provide previously unknown components of the machinery required for PRDM9-mediated recombination hotspots.

1664T **Characterization of a *RAD23* knock down UV resistance phenotype in *Tetrahymena Thermophila*** Emma J Liimatta[1], Emily M. Schmoll[2], Joshua J Smith[1] [1]Biomedical Sciences, Missouri State University, [2]Forensic Lab, St. Louis County Police Crime Laboratory

Xeroderma Pigmentosum (XP) is a recessive genetic disorder with defective Nucleotide Excision Repair (NER) caused by mutations in the XP proteins. When NER is nonfunctional the risk of skin and eye cancer increases >1000 fold because DNA adducts caused by UV radiation are unable to be repaired. There are currently no treatments or cures for the source of XP, just the presentation which is skin cancer. The NEF2 complex, composed of XPC and HR23A/B, recognizes DNA adducts and attracts NER repair proteins. XPC is widely researched yet there is little research on HR23A/B which functions to stabilize, decrease degradation, and increase efficiency of XPC. When a homolog of *HR23A/B, RAD23* (TTHERM_00013290), was knocked down in *Tetrahymena thermophila* a surprising 3-fold increase in UV resistance was observed in the *rad23* knockdowns as compared to wild type strains. This is the first time *rad23* has been studied in *T. thermophila* and previous *rad23* knockdowns in other organisms have shown UV sensitivity rather than resistance. In this study, *rad23* knockdown was confirmed by qRT-PCR, and UV resistance was confirmed by UV survivability assays. CPD dot blot immunodetection techniques assessed levels of DNA damage in UV treated *rad23* knockdown cells. TUNEL assays assessed the effect of *rad23* knockdown on apoptosis in UV treated cells. Characterization of this *rad23* knockdown UV resistance phenotype could reveal new roles that RAD23 provides in the cell. This research contributes to the current knowledge of Nucleotide Excision Repair and the molecular consequences of NER's absence in Xeroderma Pigmentosum patients. Advancing knowledge of NER, XP and RAD23 will eventually lead to the development of treatments for this genetic disease.

1665T **Cytogenomics uncovers novel rearrangements in frogs of the genus *Xenopus*** Martin Knytl[1,2], Barbora Bergelová[3], Nicola R. Fornaini[3], Halina Černohorská[4], Svatava Kubíčková[4], Tereza Tlapáková[3], Jiří Vávra[3], Vladimír Krylov[3], Ben J. Evans[5] [1]Department of Biology, McMaster University, [2]Department of Developmental Biology, Charles University, [3]Charles University, [4]CEITEC - Veterinary Research Institute, [5]McMaster University

Whole genome duplication by allopolyploidy is preceded by hybridization between two ancestral species, and is often followed by genomic rearrangements and speciation. Allopolyploid genomes have genomic compartments called subgenomes, that each derived from a different lower ploidy ancestor and frequently do not recombine with one another. Here we investigated genome structure of two allotetraploid frogs, *Xenopus borealis* and *X. laevis*, and one diploid species – *X. tropicalis*. *Xenopus* laevis is a commonly deployed model for developmental biology. Using cytogenetic approaches and chromosome-scale genome assemblies for each species, we identified inversions (i) within both L and S subgenomes that are shared by both *X. borealis* and *X. laevis* (and that occurred prior divergence of their common ancestor); and (ii) unique inversions within the L and S subgenomes in either *X. borealis* or *X. laevis*. Inversions that are specific to each subgenome of *X. borealis* or *X. laevis* suggest more recent genomic restructuring, i.e., after divergence of the most recent common ancestral lineage. Using cytogenetic approaches, we additionally identified a population-specific, female-specific inversion on the W sex chromosome of *X. borealis*. This female-specific inversion is consistent with the hypothesis that suppressed recombination surrounding sex-determining genes may be favored by natural selection. Taken together, our efforts provide a chronology of genomic restructuring following allopolyploidization in *Xenopus* and shed light on previous hypotheses regarding asymmetric subgenome evolution in this genus.

1666T **Leveraging deep mutational screening to uncover dominant Rev3 alleles as a novel synthetic lethal therapeutic strategy** Peter Stirling[1], Ecaterina Cozma[2] [1]Terry Fox Laboratory, University of British Columbia, [2]Biochemistry, University of British Columbia

Synthetic lethal (SL) therapies can exploit genome instability present in rapidly dividing cancer cells by targeting key DNA damage repair pathways. Clinically successful SL therapies, such as PARP inhibitors, act by 'trapping' the PARP protein at DNA damage sites, creating persistent cytotoxic protein-DNA lesions and limiting accessibility by redundant repair mechanisms. While PARP trapping is an effective SL therapeutic approach, it is difficult to identify inhibitors de novo with trapping properties. Cancer cells come to rely on error-prone mechanisms like translesion synthesis (TLS) for chemoresistance, making TLS polymerases a high-value cancer therapeutic target. We have leveraged high-throughput mutagenesis techniques to screen for missense variants in DNA polymerase Z (REV3) that cause dominant negative growth phenotypes. Dominant Rev3 variants in the Rev7 and Pol31 interaction interfaces have been identified and will be characterized for altered DNA binding kinetics and protein-protein interaction strength. Additional analysis of this Rev3 variant library and its effects on fitness, genetic interactions and mutation signatures are ongoing. The identification of Rev3 domains mutable to dominant forms may help to guide rational inhibitor development to selectively kill cancer cells dependent on TLS.

1667T **Coevolution of kinetochore protein Cbf2 and budding yeast centromeres** Patrick C Hecht[1], Mai Nguyen[1,2], Tia Peterson[1], Jennifer F Garcia[3], Sara J Hanson[1] [1]Molecular biology, Colorado College, [2]Computer science, Colorado College, [3]Biology, University of New England

The kinetochore is a protein complex that assembles on chromosomes at a location called the centromere, which ensures correct segregation of chromosomes during mitosis. Identifying the centromere for kinetochore assembly is an essential part of this process. Unlike other organisms, budding yeast have developed two different ways of identifying the centromere. Some species such as Saccharomyces *cerevisiae*, have a point centromere that binds to the kinetochore protein Cbf2 which then signals for kinetochore assembly. It is believed that in species with regional centromeres, kinetochore assembly happens independently of any Cbf2 function. Using bioinformatics, we identified Cbf2 homologs in a diverse range of yeast species, including those with regional centromeres. Our goal is to test the conservation of Cbf2 function in yeast with regional centromeres. We are establishing a protein-DNA binding assay that will test the ability of Cbf2 proteins in regional centromere species to bind the centromere of S. cerevisiae. In addition, we are testing the ability of these Cbf2 proteins to complement the loss of S. cerevisiae Cbf2. Through these experiments, we aim to better understand the co-evolution of centromere and kinetochore structure and function.

1668T **Inner kinetochore compositions across diverse centromere types in budding yeasts** Mai Tien Nguyen[1], Jennifer Garcia[2], Sara Hanson[1] [1]Molecular Biology, Colorado College, [2]University of New England

The kinetochore, a multiprotein structure, links centromeres to microtubules during eukaryotic cell division, ensuring accurate chromosome segregation. Centromeres are specific chromosomal regions that serve as platforms for kinetochore assembly. While functionally conserved, kinetochore composition and centromere organization exhibit diversity in eukaryotes. In budding yeasts (subphylum Saccharomycotina), centromeres vary from short, sequence-specific point centromeres to larger regional centromeres. To inventory inner kinetochore compositions in budding yeasts with varying centromere types, we developed "mign", a tool written in Python, to automate the homolog identification of 20 inner kinetochore proteins in 338 species. During the homolog identification process, certain inner kinetochore proteins in budding yeasts exhibit greater similarity to fission yeast *Schizosaccharomyces pombe*, as opposed to *Saccharomyces cerevisiae*. The resulting inventory

reveals that proteins previously recognized to be linked with point centromeres are also present in species featuring regional centromeres. Additionally, the inner kinetochore inventory in the Saccharomycodaceae family positions it as a candidate for centromere type research, given the limited knowledge of centromere types within this family. Understanding inner kinetochore compositions in relation to centromere types offers insight into the coevolution of centromeric DNA sequences and associated proteins. The data obtained here provide directions for future wet lab projects.

1669T **The role of topoisomerases in circularized chromosome strains of *Saccharomyces cerevisiae*** Mara J Stout, Melissa Mefford Department of Biology and Chemistry, Morehead State University

The genome of eukaryotic cells is characterized by multiple linear chromosomes rather than a single circular chromosome that prokaryotes typically have. Maintenance of telomeres at the end of linear chromosomes requires the ribonucleoprotein complex telomerase. Our lab is particularly interested in understanding why eukaryotic cells evolved telomeres and telomerase. To investigate the role of linear chromosomes in eukaryotes, we have successfully engineered budding yeast, *Saccharomyces cerevisiae*, with individually circularized chromosomes. In haploid strains undergoing mitotic division, the circularized chromosome strains have no obvious phenotypes compared to wildtype. However, the chromosomes must be segregated differently than wildtype strains due to the large structural difference. We propose segregation of circular chromosomes will require topoisomerases, which are important nuclear enzymes that engage in DNA replication, transcription, chromosome segregation, and recombination. To test this hypothesis, we deleted the non-essential topoisomerase I (TOP1) and III (TOP3) genes in wild-type and circularized chromosome strains of *S. cerevisiae*. Our preliminary data indicates that deletion of TOP1 and TOP3 produces growth defects for certain circular chromosomes under various conditions. To further test our hypothesis, we are growing yeast in the presence of a topoisomerase inhibitor, doxorubicin, which should mimic the effects of topoisomerase gene deletion. Together, our data will shed light on how chromosomal architecture influences chromosomal segregation and cell proliferation. In the future, we also plan to study meiotic division, hopefully elucidating that linear chromosomes are better able to undergo the vital crossing-over process in meiosis and sexual reproduction.

1670T **Characterizing the mechanism of mutagenesis in Rad5 variants in *S. cerevisiae*** Kate Jiang[1,2], Xanita Saayman[3], Jiayue Wu[4], Nicolo Tellini[3], Gianni Liti[3], Grant W Brown[1,2] [1]Biochemistry, University of Toronto, [2]Donnelly Centre for Cellular and Biomolecular Research, [3]Université Côte d'Azur, [4]University of Toronto

Mutagenesis promotes both genetic diversity and disease progression. One of the sources of mutagenesis is translesion synthesis (TLS), where lesions that block DNA replication are bypassed in an error-prone manner, allowing completion of DNA replication at the cost of introducing incorrect base pairs. *In S. cerevisiae*, the DNA repair protein Rad5 is involved in both error-prone TLS and error-free template switching, and a natural variant of *RAD5* causes increased mutation rate as a result of TLS. The mutational outcomes of Rad5-mediated TLS across the entire genome, as well as how Rad5-mediated TLS contributes to genetic diversity, remain to be understood. We performed a mutation accumulation experiment with strains carrying a template-switching deficient *RAD5* mutant, which reveals a genome-wide mutational spectrum distinct from that of WT, with the *RAD5* mutant containing more transversions. Furthermore, analysis of 2,000+ *RAD5* sequences found in wild yeast isolates revealed distribution of natural variants across the protein and conservation of functional Rad5 domains. A portion of the Rad5 ATPase domain showed presence of common variants, while the rest of the ATPase domain was largely under negative selection, suggesting different functional requirements within the same protein domain. Overall, our results highlight the importance of Rad5 in contributing to genetic diversity through both mediating the repair pathway choice and specifying distinct mutational outcomes.

1671T **Discovery of a Novel Gene that Regulates Cohesins** gurvir singh, Robert V Skibbens Biological Sciences, Lehigh University

Cohesins play essential roles in nearly all aspects of cell and development biology. First, cohesins tether together the sister chromatids in close coordination with the DNA replication fork. Second, cohesins promote high fidelity DNA repair in response to DNA damage. Cohesins also promote both chromosome condensation and gene transcription, each of which depends on cohesin ATPase-dependent DNA looping or extrusion activity.

Cohesins are regulated by at least 2 mechanisms. Eco1/Ctf7 in budding yeast (EFO1/ESCO1 and EFO2/ESCO2 in vertebrates, ESO1 in fission yeast), represents a highly conserved family of essential acetyltransferases that locks cohesins on DNA. On the other hand, Rad61 (WAPL in vertebrates) drives the dissociation of cohesins from DNA. Even though Eco1 is essential for cell viability, cells deficient in both Eco1 and Rad61are viable, but only within a very narrow temperature range. Previously, a spontaneous suppressor screen performed in *eco1 rad61* double null cells identified a number of isolates that 'revert' to robust growth at a wide range of temperatures. Whole genome sequencing of these revertants revealed a small number of gene mutations including within the open reading frame of *FDO1*. To validate that mutation of *FDO1* is the suppressor

of *eco1 rad61* double null temperature sensitive growth, we recreated both partial and full *FDO1* knockouts in the parent *eco1 rad61* double mutant strain. The results confirm that deletion of *FDO1* rescues the temperature sensitivity otherwise exhibited by *eco1 rad61* cells. This is the first report that links Fdo1 to cohesin regulation.

Fdo1 was originally identified as directing the direction of recombination during mating type switching in budding yeast. A 2 hybrid and biochemical studies further revealed that Fdo1 interacts with Fkh1 (Forkhead 1), a FOX-type transcription factor that similarly promotes donor-site preference during mating. Surprisingly, the deletion of *FKH1* had no effect on the *eco1 rad61* double null cells, nor did deletion of the FKH1 ortholog, *FKH2*. We then tested a model that Fdo1 inhibits FKH1 activity, but the overexpression of FKH1 (or FKH2) failed to revert eco1 rad61 double null cells to grow at elevated temperatures. Here, we report on additional findings that link Fdo1 to a separate transcriptional pathway and that regulates cohesion functions.

1672T **Mapping the phenotypes of single amino acid variants of the cytidine deaminase APOBEC3C with deep mutational scanning** Shamitha Aravind[1,2], Grant Brown[1,2] [1]Department of Biochemistry, University of Toronto, [2]Donnelly Centre for Cellular and Biomolecular Research

The apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (*APOBEC3*) family consists of seven paralogous cytidine deaminase genes (A – D and F – H). Cytidine deaminases cause cytidine to thymidine transition mutations when left unchecked by DNA repair mechanisms. Members of the *APOBEC3* family support innate immunity by hypermutating viral and/or retrotransposon DNA. In addition to acting on pathogens and mobile elements, some APOBEC3 proteins deaminate genomic DNA. Emerging evidence implicates *APOBEC3C* (*A3C*) in roles outside of innate immune support; however, the multiple functions of this protein have not been comprehensively investigated. Wild-type A3C has relatively less deaminase activity compared to a naturally occurring variant, $A3C_{S188I}$, which dimerizes more readily. The existence of a protein variant that is less frequent and more active than wild-type A3C reflects the value of phenotyping a large pool of A3C missense mutants in an unbiased manner. I am characterizing the sequence-function relationships for A3C through deep mutational scanning (DMS) in the budding yeast *Saccharomyces cerevisiae*. DMS probes protein function through the analysis of all possible single amino acid variants, allowing the construction of high resolution structure-function maps. By subjecting a library of A3C missense protein variants to multiple assays, I will identify residues that are critical for the cellular function and dimerization of A3C. My approach will provide key insights into the structural characteristics of A3C that underlie its functions.

1673F **The sexually dimorphic recombination landscape in *C. elegans* meiosis coordinates with sex-specific germline gene expression** Zachary Bush[1], Alice S Naftaly[1], Devin Dinwiddie[1], John S Conery[2], Kenneth Hillers[3], Diana E Libuda[1] [1]Biology, University of Oregon, [2]University of Oregon, [3]Biological Sciences, California Polytechnic State University, San Luis Obispo

Crossover recombination events repair DNA damage, create genetic diversity, and ensure the accurate segregation of chromosomes during meiosis in most organisms. In many species, the genomic distribution of crossovers is nonrandom and varies between sexes. While many species evolved kilobase-scale “hotspots” where crossover events are more likely to form, the *Caenorhabditis elegans* genome lacks such hotspots and crossovers are biased to form across megabase-scale domains on the terminal thirds of each chromosome. Further, genetic and cytological experiments indicate *C. elegans* spermatogenesis exhibits higher crossover frequencies in comparison to oogenesis, however, the mechanisms that lead to this sexually dimorphic crossover regulation are poorly understood. To define and analyze these sexually dimorphic recombination landscapes at high resolution across the entire genome, we performed whole-genome sequencing and high-resolution recombination mapping of single worms with recombinant chromosomes from individual meiotic products of either spermatogenesis or oogenesis. We find that the fine-scale distribution of crossovers is sexually dimorphic on chromosomes I, II, and III, and that the rate of crossing over is elevated in spermatocytes. The rate of double crossover events is 4.7 times higher in developing sperm and regulation of inter-crossover distance appears less stringent. The frequency of double crossover events, however, is not shared between all chromosomes, as spermatocyte chromosomes vary from 1.5-7.6% on the autosomes. Further, we define the sequence-level genome annotations and sex-specific chromatin environments associated with the sexually dimorphic crossover distribution. Among our findings, we identify a strong association of ncRNAs, promoters, enhancers, and untranslated regions (UTRs) of genes in genomic regions with sexually dimorphic crossover frequencies. We also detect an enrichment of crossovers in genomic regions associated with actively expressed genes in the male versus hermaphrodite germline. Overall, these studies provide a framework for high-resolution crossover mapping in individual *C. elegans* genomes and reveal potential regulators of the sexually dimorphic crossover distribution.

1674F **Transgenerational Screening of Intrinsically Disordered Proteins for Genes Involved in Meiosis in the Nematode *C. elegans*** yvan doctorovich[1], William McFadden[2], Judith Yanowitz[1] [1]Magee-Womens Research Institute, [2]Department of Pediatrics, Emory University School of Medicine and Children’s Healthcare of Atlanta

Intrinsically disordered regions (IDRs) play critical functions in proteins without having a fixed structure. Unlike most proteins,

which fold into specific conformations to carry out their functions, IDRs are flexible and dynamic, lacking a fixed structure even in the absence of molecular interactions. Despite this, IDRs have specific amino acid compositions and properties that are essential for protein function. At the same time, they have different evolutionary constraints on them because they need only retain charge and not a specific amino acid composition. In this regard, they may appear to be rapidly evolving while in fact retaining function. Our lab has worked on several meiotic proteins that are highly disordered, leading us to consider whether this class of proteins may reveal novel germline functions.

To test this hypothesis, we compiled a comprehensive list of the highly disordered proteins in the worm model, *Caenorhabditis elegans*. We learned this way that they tend to be basic and nuclear, which could support their function as transcription factors. We then knocked them down individually using RNA interference (RNAi) and analyzed effects on fertility by examining the subjects' phenotypes *in vivo* over three generations and DAPI staining the adults after three generations of exposure to look for abnormalities *ex vivo* in the gonadal or embryonic development. This screen was realized on the RNAi sensitive strain *lin-35(ea86)* and the positives were confirmed in the wild-type strain N2.

25 of the 110 genes analyzed caused a reproductive phenotype. The results of this study highlight the significance of these understudied proteins and their potential importance in reproduction in *C. elegans*. It also emphasizes the importance of continued research into IDRs, which could lead to the discovery of novel targets for therapeutic intervention in human infertility.

1675F **Natural variation in mutagen tolerance among *C. elegans* wild isolates** Sophia Tintori, Matthew V Rockman New York University

All living cells are continuously challenged by exogenous, endogenous, and spontaneous DNA damage. While conserved DNA damage response keeps organisms safe broadly, natural variants in these mechanisms can carry profound consequences for cell biology, health, and evolutionary genomics. In this study we use wild nematode strains to study the mutagen tolerance and DNA repair variants that exist in natural populations, and the cell biological and mutagenic consequences of those variants.

We have developed a highly sensitive assay to identify which wild strains are naturally more or less tolerant to different DNA damaging agents during chronic exposure over multiple generations. We have applied this assay to 35 strains across two species: *C. elegans* strains that were previously collected from around the world, and *O. tipulae* that we collected from the Chornobyl Exclusion Zone, a radioactive landscape that we hypothesized may have already selected for mutagen tolerance. We have tested these strains for their diverging sensitivities to a range of DNA damaging chemicals, and have identified phenotypic outlier strains. We have performed bulk segregant analyses with hybrid populations of these outlier strains, identifying alleles of interest for mutagen tolerance phenotypes.

1676F **Pink1 represses apoptosis and allows proper morphogenesis after exposure to ionizing radiation in *Drosophila melanogaster*** Lauren M Orr, Hannah Golding, Ellie O›Brien, Joshua Siauw, Tin Tin Su Molecular, Cellular and Developmental Biology, University of Colorado Boulder

There is accumulating evidence of a direct relationship between the DNA Damage Response and autophagy, but the overall picture lacks clarity. To elucidate this relationship, we conducted a focused RNAi screen for autophagy regulators in the context of ionizing radiation (IR). Using morphological disruption of the compound eye of *Drosophila melanogaster* as a readout, we identified two genes of interest: ref(2)P (*Drosophila* p62/SQSTM1) and Pink1. Both genes are necessary for successful mitophagy, a subset of macro-autophagy. We found that RNAi knockdown of these genes resulted in defective eye morphogenesis when larvae were irradiated in the 3rd instar and the resulting adult eyes were examined. Cell biological analysis showed that Pink1 knockdown did not alter cell proliferation or cell cycle checkpoints after irradiation but increased apoptotic activity post-IR in 3rd larval instar eye discs. This result was reproduced with Pink1^{5} loss-of-function mutants that showed a more pronounced effect on apoptotic activity, specifically in the G1 arrested cells of the morphogenetic furrow. These results suggest that mitophagy might be important for cells in G1 to resist IR-induced apoptosis and ensure proper eye morphogenesis during recovery from radiation damage.

1677F **An Unbiased Screen for Vulnerabilities of Cells with Centrosome Amplification** Jane E Blackmer, Erin Jezuit, Ruth Montague, Archan Chakraborty, Don Fox Duke University

Centrosomes are microtubule organizing centers in animals, coordinating bipolar chromosome segregation during mitosis. Centrosome amplification (CA), a common cancer phenomenon, can pose a problem to the dividing cell as this drives multipolar division, aneuploidy, and often cell death. Usually, cells can compensate for CA by clustering extra centrosomes to two spindle poles. In the cancer therapy field, treatment with anti-clustering agents have attempted exploit CA in cancer and promote multipolar division-induced tumor cell death. However, anti-clustering agent treatment has proven to be

only moderately effective in cancer, suggesting that additional, clustering-independent mechanisms operate in cells with CA. *Drosophila* rectal papillar cells serve as an excellent system to study multipolar division with extra centrosomes, as these cells seldom cluster centrosomes and are highly tolerant of CA and multipolar division.

To identify factors that enable papillar cell survival of multipolar division, we performed an unbiased EMS screen in the *Drosophila* papillae. Our screen examined 642 recessive lethal mutations that we used to generate homozygous mutant clones in developing papillae through mitotic recombination. Our screen exploited the lethality of animals with compromised papillae to a high salt diet, as papillae are required for ion balance. Upon generating mitotic clones in papillar tissue, we screened for mutations that resulted in high salt diet lethality in combination with experimentally induced CA. From this screen, we have identified 26 mutant lines that reproducibly decrease papillar cell survival upon CA. Follow up analysis has placed two of these lines in a complementation group, suggesting our screen approached saturation. At my poster, I will reveal the identity of the gene in this complementation group, which encodes a protein that is orthologous to a target of an FDA approved seizure drug. Our screen is uncovering novel mechanisms of cell survival after CA and promises to identify novel cancer therapy drug targets.

1678F **Unveiling of Meiotic Aneuploidy through Targeted Ovarian Genes** Sarah Abrahem, Brianna Miller, Huda Zaman, Jessica Fellmeth Millersville University of Pennsylvania

The leading genetic cause of infertility is aneuploidy. Aneuploidy is when a cell has too many or too few chromosomes. When this occurs in gametes such as sperm or egg, it can result in infertility or birth defects. In somatic cells, aneuploidy is a hallmark feature of most cancers. Fertilization is where the haploid sperm and the egg fuse together to form a diploid zygote which contains the egg and sperm chromosomes. Then the zygote undergoes mitosis to develop the embryo. Meiosis is a special two-part type of cell division used to create gametes. The first part of meiosis (MI) is the origin for many of these chromosome errors, and this occurs more frequently in eggs than in sperm. These errors happen through nondisjunction of homologous chromosomes in meiosis (MI) in females. It occurs when two homologous chromosomes are pulled toward the same pole by spindle fibers rather than being separated creating an aneuploid egg that gives rise to an aneuploid embryo. The widely accepted source of these errors is the “Long Prophase Pause” which occurs only in oocytes (immature eggs). When females are born, all their oocytes are arrested in the early stages of prophase I of meiosis, this pause lasts until the egg is ovulated which can be 14-40 years later. All the proteins that are on the chromosomes during this period undergo an aging affect which can result in problems with chromosome segregation later.

This project is part of a larger screen attempting to identify new genes involved in meiotic chromosome segregation. Target genes were selected based on expression data from FlyAtlas2 and availability of RNAi stocks from TRiP. I aimed to use these shRNA’s to investigate eight distinct genes that are highly expressed in Drosophila ovaries. According to our preliminary studies, one of the strains has an increased amount of aneuploidy indicating a possible meiotic function. The gene targeted by this shRNA is CG7794 which is a putative protein coding gene on chromosome 3. It is predicted to be a structural component of the cytoskeleton and involved in organization of the spindle in mitosis. It’s role in cell division has not been studied and we look forward to collecting more data regarding the function of this gene in chromosome segregation.

1679F **How chromosomal inversions suppress meiotic recombination: Testing the breakpoint interference hypothesis in *Drosophila melanogaster*** Spencer A Koury Department of Biological Sciences, Auburn University

Recombination suppression in chromosomal inversion heterozygotes is well-documented but poorly understood. In *Drosophila melanogaster*, recombination suppression extends far outside inverted regions where there are no intrinsic barriers to normal chromosome pairing, synapsis, double-strand break formation, or segregation of crossover products. Even though formation of dicentric chromosomes via anaphase I bridges is often invoked to explain suppression inside inverted regions, empirically this mechanism only accounts for 10-20% of the chromosome-wide recombination phenotype in *D. melanogaster*. Thus, 80-90% of the fundamental phenotype of inversion heterozygosity remains unexplained a century after its first discovery. The breakpoint interference hypothesis addresses this knowledge gap by proposing heterozygous inversion breakpoints induce local discontinuities in the synaptonemal complex and, therefore, possess chiasma-like properties. This hypothesis predicts recombination suppression extends from breakpoints in a process analogous to crossover interference, which is qualitatively consistent with suppression extending to both inverted and uninverted chromosomal regions. Here, I develop quantitative predictions for this hypothesis building upon probabilistic models of crossover interference with gamma-distributed inter-event distances. Using *D. melanogaster*, I test these predictions with my own experimental data (>40,000 meioses) on crossing-over outside 4 inversions of the 3rd chromosome. Next, I test predictions for inside inverted regions with published data (>100,000 meioses) from 5 different inversions embedded in metacentric compound X chromosomes. Finally, I compile recombination data for 17 inversions occurring along acrocentric X chromosomes (>300,000 meioses) to test anecdotal claims that distal inversions are stronger crossover suppressors. The results demonstrate that the breakpoint interference hypothesis

accurately predicts suppression outside inversions, substantially improves predictions of suppression inside inversions, and reveals distal inversion are indeed stronger suppressors due to systematic variation in crossover interference. In sum, the breakpoint interference hypothesis provides a mathematical framework for integrating distance-dependent effects of structural heterozygosity on crossover distribution and highlights the potential for integrating statistical theory with biophysical models of crossover patterning along the chromosome.

1680F **Meiotic Crossover Designation and Interference in Drosophila Involves ATR-Dependent Phosphorylation of Mei-218** Susan McMahan, Colette Anikwue, Jeff Sekelsky University of North Carolina at Chapel Hill

The mechanism of crossover interference has been a mystery since Sturtevant described it in 1913. Meiotic recombination begins with DNA double-strand breaks (DSBs), then repair in the context of the synaptonemal complex (SC) culminates with most DSB sites becoming noncrossovers but at least one per bivalent or chromosome arm being selected to be a crossover. If more than one becomes a crossover, they are widely spaced. The discoveries that SC has liquid-like properties and that meiotic RING finger proteins move within the SC and accumulate at sites designated to become crossover led to a model in which coarsening (Ostwald ripening) of RING finger proteins leads to one site or widely separated sites being fated to become crossovers.

A key question is how RING finger protein accumulation can promote crossover repair. We propose that in Drosophila this involves stabilization of the mei-MCM complex to block the anti-crossover activity of Blm helicase, allowing the meiotic Holliday junction resolvase to make a crossover. We propose that stabilization involves phosphorylation by Mei41 (ATR kinase, which we previously showed to be required for interference). Mei218 has an intrinsically disordered region (IDR) in the N-terminus that is under positive selection. We identified putative ATR phosphorylation sites and mutated them the endogenous gene from S/T to A (non-phosophorylatable) or D (potentially phosphomimetic) in numerous combinations, then measured crossovers and interference. A striking finding not previously reported is that in a *mei-218* null mutant, where crossovers are reduced >90%, there is high negative interference (far more double crossovers than expected). Changing a cluster of 3 S/T residues near C-terminus of unstructured region to A made a null allele, though each singly change had no effect. Changing the 5 more N-terminal residues created a hypomorphic allele, and changing all 8 made a weaker hypomorph. Changing these sites to D also produced a series of different allele strengths, with 3D, 5D, and 8D having progressively stronger (though not null) phenotypes.

Our results support the hypothesis that phosphorylation of Mei218 by Mei-41 regulates both crossover designation and interference but suggest that dephosphorylation may also play a role and different sites may have different effects.

1681F **A mutation in the CAL1 binding site of CENP-C shows that CAL1 is not necessary for prometaphase function of CENP-C in meiosis** Eve Jeffries-Walters[1], Jadyn Koser[1], Brandi Wolfinger[1], Madison Holmes[2], Kim S McKim[3], Jessica Fellmeth[1] [1]Biology, Millersville University, [2]Millersville University, [3]Genetics, Rutgers, The State University of New Jersey

Aneuploidy is the leading genetic cause of female infertility. These errors largely originate from nondisjunction (NDJ) of homologs in meiosis I (MI). Female meiosis is uniquely error prone due to a long pause in prophase. Females are born with their oocytes arrested in prophase of MI and the chromosomes don't segregate until ovulation some 14-40 years later. CENP-C, which acts as the bridge between the centromere (chromosome) and the kinetochore (machinery for segregation), is one protein necessary for maintaining chromosome structure through this pause. Previous work from our lab has shown that CENP-C activity during the pause is dynamic and loss of this movement results in aneuploidy. This activity is distinct from mitosis and therefore of special interest. We have created a HA-tagged CENP-C mutant (CENP-C^{FR}) that has a single base pair substitution in the CAL1 binding site. CAL1 is a CENP-A chaperone necessary for CENP-A and CENP-C recruitment to centromeres. We have previously shown using CAL1 RNAi that CAL1 isn't necessary for the dynamic prophase loading of CENP-C. Using tissue specific promoters, we expressed CENP-C^{FR} in *Drosophila* oocytes before or during the pause. We hypothesized that the latter group will appear normal at metaphase I, but the former will exhibit defects. As expected, we observed increased aneuploidy in oocytes expressing CENP-C^{FR} before the pause, similar to a loss of CENP-C. However, flies expressing CENP-C^{FR} after the pause were completely sterile. This does not support our hypothesis, but is similar to the phenotype observed when wildtype CENP-C is expressed with the same promoter. The wildtype-expressing oocytes have been shown to be completely normal at metaphase I, so we believe the defect is during embryogenesis. If this holds true for CENP-C^{FR}, we expect to see a wildtype phenotype at metaphase I. When expressed only in early prophase, the CENP-C^{FR} mutant oocytes displayed levels of NDJ higher than CENP-C knockdown oocytes indicating a possible dominant effect. Cytological studies are ongoing as well as studies using a similar promoter with reduced expression to hopefully avoid the sterility phenotype. This data supports the conclusion that CENP-C activity during the prophase pause is independent of CAL1 which is a completely novel activity for CENP-C. Our aim for this study is to continue to shed light on the mechanisms of CENP-C function during meiosis. Allowing a deeper understanding of the behavior of the centromere, kinetochore, and chromosomes

during the long prophase pause in female meiosis.

1682F **Whole-genome approaches to understanding meiotic recombination mechanism and regulation** Carolyn Turcotte, Jeff Sekelsky Genetics and Molecular Biology, University of North Carolina at Chapel Hill

During meiosis, crossovers between homologous chromosomes ensure proper chromosome segregation. Crossovers are formed by repairing double-strand DNA breaks (DSBs) via homologous recombination (HR). The number and spatial arrangement of crossovers ("crossover patterning") is tightly regulated, and failures in this regulation can lead to aneuploidy and miscarriage. The mechanism behind this regulation and the primary defects that contribute to aneuploidy remain poorly understood. I use a two-pronged approach to study these phenomena in *Drosophila*: 1) mapping recombination events in progeny derived from aneuploid oocytes, and 2) finely analyzing HR products using whole-genome sequencing.
In the first approach, I use a genetic trick to exclusively collect progeny derived from diplo-*2* oocytes (oocytes that experienced chromosome *2* nondisjunction). Preliminary data from whole-genome sequencing of these progeny indicate that many nondisjunction events are the result of failure to properly segregate sister chromatids in MII. Intriguingly, all crossovers observed in progeny thus far were single crossovers that occurred in the middle of chromosome arms, which is typical of normal crossover patterning in *Drosophila*.
In the second approach, I analyze heteroduplex DNA (hDNA), double-stranded DNA in which each strand comes from a different DNA molecule, to better understand recombination mechanism. The classical meiotic HR model indicates that a crossover is formed via a double Holliday junction (dHJ), a structure in which two DNA molecules are linked via criss-crossing of their strands at two adjacent sites. In this classical model, ligated dHJs give rise to all crossovers and generate one of two possible hDNA signatures by being cleaved on all four strands. The model predicts that both signatures are equally likely, yet only one of the hDNA signatures has been observed experimentally. Our lab has mapped hDNA at recombinants of a test locus in Drosophila melanogaster, but redefining the meiotic recombination model requires much more extensive analysis of hDNA than is possible with this methodology. To overcome this obstacle, I am pioneering "hetSeq", a whole-genome sequencing technique to detect hDNA from meiotic products, to continue redefining this model. Shedding light on HR mechanism and crossover patterning is critical to our understanding the factors that contribute to proper chromosome disjunction.

1683F **Control of D. mel Centromere Effect: Genetic or Spatial?** Nila M Pazhayam[1], Jeff Sekelsky[2] [1]Genetics and Molecular Biology, UNC Chapel Hill, [2]Biology, UNC Chapel Hill

Crossing-over between homologous chromosomes is a critical part of meiosis that promotes proper chromosome segregation, thereby forestalling miscarriages and chromosomal disorders such as Down syndrome. Meiotic crossovers (COs) are formed from programmed double-strand breaks (DSBs) that undergo homologous recombination. Although DSBs are distributed throughout the chromosome, CO placement is intricately patterned. One such patterning event is the centromere effect (CE) which ensures CO exclusion in pericentromeric regions. Although crucial to the meiotic cell, the mechanisms driving the CE are poorly understood. To address this gap in knowledge, I am studying the CE using *Drosophila melanogaster* as a model system.

The pericentromeric region of *D. melanogaster* isn't homogenous, instead consisting of two distinct types of heterochromatin; highly-repetitive alpha heterochromatin and less-repetitive beta heterochromatin. A previous study from our lab has shown that the CE is distinct in the two types of heterochromatin, with a complete suppression of COs observed in alpha heterochromatin while beta heterochromatin/proximal euchromatin display a distance-dependent suppression. To further characterize CO control in these regions, I will be mapping proximal COs to either alpha or beta heterochromatin in two classes of mutants - genetic and structural – that have previously been shown to weaken the CE. Genetic mutants include mutants such as *mei218* and *rec,* and structural mutants include genes important for heterochromatin formation such as *Su(var)309* and *Su(var)307*. As the question of whether the CE is under genetic or structural/spatial control remains unanswered, this project aims to investigate how centromere-proximal CO formation differs in different classes of mutants. It is possible that in the *Su(var)* mutants being used in our study, we observe COs in the highly-repetitive alpha heterochromatin - where meiotic crossing-over has never been observed before – while proximal COs in the genetic mutants are limited to beta heterochromatin/proximal euchromatin. Ultimately, whatever pattern of proximal CO formation we observe in the two types of heterochromatin in these mutants will shed light on the mechanisms of CO control that are at play in the pericentromere, thereby providing insight into the *Drosophila* centromere effect.

1684F **Elucidating the role of Polo kinase regulation in suppressing drive of the B chromosomes** Kaylah B Samuelson[1,2], Ryan Gado[1], Allison Gardner[1], Stacey L Hanlon[1,2] [1]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

Recently, B chromosomes were discovered in a single stock of *D. melanogaster* that carries a null mutation in *matrimony* (*mtrm*^{126}) held over a third chromosome balancer (*TM3, Sb Ser*). This genotype enables the B chromosomes to be transmitted through female meiosis at a higher-than-expected frequency, a phenomenon known as meiotic drive. Mtrm

normally works with Polo kinase (Polo) in a 1:1 genetic ratio to regulate chromosome segregation, leading us to speculate that the maintenance of this ratio is important to suppress the drive of the B chromosomes. To test this, we genetically altered the ratio of *mtrm* and *polo* using null alleles and found that B chromosome transmission frequencies decrease as the genetic levels of Mtrm increase relative to Polo, suggesting that a high Polo:Mtrm ratio is necessary to promote drive of the B chromosomes. Since the direct interaction of Polo and Mtrm has been previously shown to be essential for proper chromosome segregation, we also tested if this interaction is necessary to suppress the drive of the B chromosomes. We found that B chromosome transmission is still high in a $mtrm^{126}/TM3$ background that expresses a mutant transgene of Mtrm that cannot bind Polo ($mtrm^{T40A}$), indicating that the direct interaction of Mtrm and Polo is necessary to suppress drive. Our results show that the regulation of Polo by Mtrm promotes drive suppression, leading us to explore if other pathways that regulate Polo influence the drive of the B chromosomes. We are currently assessing B chromosome transmission in a $mtrm^{126}/TM3$ background that expresses a hypermorphic transgene of Greatwall (Gwl), a meiotic and mitotic kinase that antagonizes Polo. Overall, our investigation will illuminate how the regulation of Polo kinase influences the meiotic drive of the B chromosomes.

1685F **Homologous Recombination during CRISPR-Induced DNA Double-Strand Breaks** Reese L Perini, Evan Dewey, Jeff Sekelsky Biology, UNC Chapel Hill

Homologous recombination repair (HR) is a vital DNA repair mechanism essential for organismal growth and survival. Misregulation of HR can lead to loss of heterozygosity (LOH) in tumor suppressor genes, potentially facilitating the development of genetic diseases such as cancer. LOH occurs when a functional tumor suppressor allele on one homologous chromosome is lost, resulting in homology for a nonfunctional tumor suppressor. Homologous loss of tumor suppressor function promotes cancerous transformation. Most DNA double-strand breaks are resolved into noncrossover (NCO) genetic products by HR. Double-Holliday junction (dHJ) resolution, a type of HR with an intermediate DNA configuration called a dHJ, leads to rarer crossover (CO) products. COs formed during mitosis can lead to dangerous exchanges between homologous chromosomes and LOH. In order to test proposed models and decipher precise mechanisms of dHJ resolution, we reconstructed CRISPR/Cas9 DNA repair products in *Drosophila melanogaster* using PCR and Oxford Nanopore sequencing. Our findings support proposed models of dHJ cleavage. We also found products that are not readily explained by current models. These unique products characterize exceptions to our proposed models of dHJ cleavage, helping us to better understand this recombination mechanism. Results also provide insights into how COs form in general, and how HR works in a CRISPR/Cas9 context, which can lead to more efficient and successful gene-editing strategies.

1686F **Usage of Clamps during Homologous Recombination Repair of Large Gaps in Drosophila** Meaghan Dineen, Bridget Walker, Daniel P. Kane Le Moyne College

Proper repair of DNA damage events is necessary to prevent genomic mutations and the development of diseases and disorders. DNA double-strand breaks (DSBs) are problematic, compared to single-strand breaks, as template for recovery of any lost sequence is not immediately evident. Repair by end joining is error-prone and can result in loss of flanking sequence to the break site. More accurate repair by the homologous recombination (HR) pathway involves synthesis of new DNA to recover any lost information after template invasion, using a sister chromatid or homologous chromosome, and D-loop formation. In Drosophila, the $P\{w^a\}$ assay uses the excision of a transposable element to generate a single 14-kilobase gap, thereby requiring a large amount of nascent synthesis for full recovery. With this assay, we have previously shown that both replicative and translesion synthesis DNA polymerases compete in the repair process, with replicative polymerases being utilized in longer synthesis tract lengths and translesion polymerases in shorter repair synthesis. In our current research, we hope to determine how the selection of these different polymerase types for utilization at sites of DSBs is coordinated. Roles of polymerase clamps are best known in replication and some repair processes, but are not as clear for homologous recombination in gap repair. By examining *hus1* mutants (part of the 9-1-1 clamp complex) and *PCNA2* mutants we hope to elucidate upstream coordination of polymerase-type choice for this nascent DNA synthesis at HR D-loops. Preliminary analysis suggests that, like polymerases, different clamps are utilized for short versus long synthesis tract lengths, demonstrating the need for clamps to recruit and aid polymerase processivity in large-gap HR repair.

1687F **Mechanism of Somatic Chromosome Pairing in *Drosophila melanogaster*** Makenna Johnson, Kent Golic University of Utah

The ability to pair homologous chromosomes in meiosis is fundamental to sexual reproduction and is achieved through various strategies, such as induced double-strand breaks and recombination. *Drosophila melanogaster* and other Diptera are unique in their ability to pair and maintain pairing in somatic cells, even without recombination. The means by which pairing initiates and is maintained has been of intense interest, but definitive models have yet to emerge. Current models hypothesize that initiation can happen in one of two ways: 1) random interactions of the chromosomes, or, 2) initiation at specific sites, such as telomeres, centromeres, or “pairing sites”. After initiation occurs, these homologous sequences are then proposed to ‘zipper’

together until full homolog pairing is achieved. To investigate the mechanism of somatic chromosome pairing we developed a method that uses heterozygous inversions to measure the degree of homolog pairing. *X* chromosome inversions generated by FLP-mediated recombination revert at a high rate in the presence of FLP, a process that requires pairing with an un-inverted homolog. Inversion loop pairing brings inversion breakpoints into close proximity to facilitate recombination and reversion. By deletion analysis we identified a locus in polytene division 11 on the *X* chromosome that acts as pro-pairing sites and a separate locus that acts as anti-pairing site. These sites do not affect autosomal pairing, indicating that they are likely acting structurally, rather than encoding genes involved in pairing. Additional analysis and results suggest that there are many pro- and anti-pairing sites along the length of the chromosome that either initiate or maintain homologous pairing in somatic cells.

1688F **Loss of *Nemp1* triggers oocyte loss by activating DNA Damage Pathway** Bilal A Hakim[1], Yonit Tsatskis[2], Won Ha[2], Andrea Jurisicova[2], Helen McNeill[1] [1]Developmental Biology, Washington University School of Medicine, [2]Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital

NEMP1, a conserved protein located in the nuclear envelope, is crucial for the process of oogenesis across various species such as flies, worms, fish, and mice. During oogenesis, a developmentally competent female gamete goes through a series of morphological and functional phases from the primordial to the ovulatory follicle. The ovarian lifespan, and ultimately the fertility of an individual, is in part determined by the quantity of primordial follicles in the ovarian reserve. This reserve possesses all the oocytes an individual will have during their reproductive lifespan. *Nemp1*^{KO} mice are viable, but females are severely subfertile with drastically reduced ovarian reserve. The remaining oocytes have poor developmental competence with defects in chromatin organization. Examination of P10 *Nemp1*^{em1Hmc} ovaries revealed an extensive loss of primordial follicles, which was also detected at the P1-P4 stages. Excessive cell death was observed in the P1-P4 ovaries compared to the P10 ovaries, suggesting the reduced ovarian reserve of *Nemp1* mutants may be caused by apoptosis. Embryonic mouse ovarian surface spreads at E17.5 showed 36% *Nemp1* mutant oocytes have a broad patch of □H2aX as compared to 11% in the WT type. Increased incidence of aneuploidy was also observed in pachytene-stage *Nemp1* mutant oocytes. Analysis of meiotic stages at P0 also demonstrated a delay in Meiotic I progression in *Nemp1* mutant oocytes. Since NEMP1 has a conserved role in fertility, we believe Drosophila can be helpful for studying these NEMP1-specific defects. As reported earlier from the lab, Drosophila Nemp null animals are also sterile, with a significantly reduced ovary size and severe germ cell loss. Closer examination of ovaries upon Nemp knockdown revealed phenotypes consistent with activation of DNA damage response (DDR). To investigate the role of the DDR in the absence of Nemp, we used RNAi knockdown of DDR pathway components such as Tefu (Atr, Chek1, Chek2, and p53. Remarkably, loss of ovary size and egg laying caused by germline expression of Nemp RNAi was partially rescued by simultaneous reduction of Tefu or Chek2, demonstrating that a DNA damage checkpoint was triggered upon Nemp loss. The ovarian reserve in *Nemp1*^{KO} mice was also partially rescued in *Nemp1*^{KO}*Chek*^{KO} double mutants, indicating *Nemp1* loss activates a DNA Damage checkpoint in mammals.

1689F **Uncovering the effect of ZMPSTE24 deficiency on genome integrity** Alannah DiCintio, Alan Waldman University of South Carolina

Restrictive Dermopathy (RD), is an autosomal recessive rare genetic disorder that is neonatal lethal. If children are liveborn, they usually do not make it through the first week of life due to pulmonary insufficiency. RD is commonly caused by insertion mutations that produce premature termination codons in both copies of the *ZMPSTE24* gene. Interestingly, ZMPSTE24 levels are also believed to decrease naturally during the aging process. ZMPSTE24 is a protease that plays a key role in the post-translational processing of lamin A, an essential protein in a structure called the nuclear lamina that plays a critical role in a variety of nuclear functions. The form of lamin A produced in the absence of ZMPSTE24 is permanently farnesylated and methylated. Previous studies from our lab and other groups have shown that other mutant forms of lamin A that are incompletely processed disrupt the repair of DNA double-strand breaks (DSBs) and cause a decreased ability to maintain genomic integrity. Changes in DNA repair have also been associated with the normal aging process. To enhance our understanding of both the biology of aging as well as RD, we are investigating the effects of ZMPSTE24 deficiency on DNA repair and damage accumulation. To do so we have stably transfected a DSB repair reporter substrate into the genome of a HeLa cell line that expresses ZMPSTE24 and into a second HeLa cell line in which ZMPSTE24 expression had been knocked out by CRISPR/Cas9. A DSB can be induced within the substrate through exogenous expression of endonuclease I-SceI, and DSB repair events occurring via either homologous recombination (HR) or non-homologous end-joining (NHEJ) are recoverable by genetic selection. Repair by HR is an accurate and templated form of repair, while NHEJ is an error-prone non-templated rejoining of DNA ends that may lead to deletion or insertion mutations. Our work aims to reveal the impact of ZMPSTE24 deficiency on repair pathway choice. We are also investigating DNA damage accumulation in cells that express ZMPSTE24 and ones that do not by directly visualizing γH2AX foci using immunofluorescence approaches. Results to date indicate increased damage in the absence of ZMPSTE24. We present our ongoing studies using our model system to investigate the effects of ZMPSTE24 deficiency on genome integrity.

1690F **The DNA structural landscape at the centromere and pericentromere** Lydia Gutema, Jitendra Thakur Biology, Emory University

Centromeres are chromosomal loci that bind to spindles to facilitate chromosome segregation and ensure faithful inheritance of genetic information in daughter cells. Issues during centromere formation can result in the gain or loss of genetic material in daughter cells, which can have deleterious effects on human health. DNA sequences at the endogenous centromeric locus are highly repetitive, and while there is some sequence similarity, centromeric sequences at different chromosomes are distinct from each other. Despite the lack of conserved sequences, centromeric proteins are recruited to correct site during each round of cell division. How centromeric DNA encodes the centromere in the absence of conserved sequences is not well understood. Additionally, when there is a loss of the endogenous centromeric sequences, ectopic centromeres can form to maintain inheritance of the chromosome, and these mislocalized centromeres can be seen in cancers and developmental disorders. Ectopic centromeres have a non-random distribution across the genome, which raises interesting questions about how centromeres are assembled at these sites. However, conserved features of the DNA or chromatin at ectopic centromeres and at the endogenous centromeres have not been discovered yet. We hypothesize that DNA structures help play a role to specify the sites of centromeres formation. Our early data shows a distinct change in patterns of certain DNA structures within the active centromeric region and the surrounding inactive pericentromeric regions. Our future work will assess how these differences contribute to centromere specification and how proteins at the centromere and pericentromere establish distinct changes in the structural landscape. This work will allow us to address longstanding questions in the field about how the localization of centromeres is determined in humans, and better understand how unique DNA structures play a role in cellular function.

1691F **Characterization of the Overexpression of RecA Homologs *DMC1* and *RAD51* in *Tetrahymena thermophila*** Jianna M. Cox, Joshua J Smith Biomedical Sciences, Missouri State University

Two RecA homologs, *DMC1* and *RAD51*, work to repair DNA double-strand breaks (DSBs) within the cell through the recombination of homologous sections of DNA. While *DMC1* works to repair programmed DSBs through meiotic recombination, *RAD51* functions to repair both meiotic and non-meiotic DSBs, the latter being repaired through the repair process of homologous recombination. Double-strand breaks can occur through various endogenous and exogenous agents, and when not repaired can cause harmful genomic rearrangements and mutations. Chemotherapeutics are an exogenous agent that work to form DSBs in cancer cells, attempting to inhibit the cells from further spread. However, a hyper-recombinant phenotype is often seen in cancer cells due to the overexpression of *RAD51*. This leads to drug resistance, the persistence of cancers, and an overall poor patient outcome. To work to prevent this issue, further research is being conducted on the function of *RAD51*. In the model organism *Tetrahymena thermophila*, an amacronuclear phenotype is observed only at high temperatures, such as 35 °C, when *RAD51* is overexpressed. Further evidence shows that this phenotype is more affected from the change in temperature rather than only the temperature itself. This is due to a complication in the elongation of the macronucleus, and due to the continuation of DNA synthesis, results in a macronucleus containing up to 5 times the normal genetic content. When *DMC1* is overexpressed, no notable phenotype is observed. When studying the relationship between the mutation and survivability of cells when treated with UV and MMS, no strong correlation was observed.

1692F **DNA double-stranded break and new telomere formation during *Ascaris* programmed DNA elimination** Brandon Estrem[1], Jianbin Wang[1,2] [1]Biochemistry & Cellular and Molecular Biology, University of Tennessee Knoxville, [2]UT-ORNL Graduate School of Genome Science and Technology, University of Tennessee Knoxville

Organisms must maintain the integrity and the full complement of their genome in all cells. However, some organisms undergo a process of DNA loss known as programmed DNA elimination (PDE). In metazoans, PDE often occurs during early embryogenesis in all pre-somatic cells, generating a reduced somatic genome. The human and pig parasitic nematode, *Ascaris*, is a model for studying PDE. In *Ascaris*, DNA double-strand breaks (DSBs) are generated at 72 specific genomic loci, fragmenting the 24 germline chromosomes into 36 somatic chromosomes. Broken ends are healed with *de novo* telomere addition to protect the new chromosome ends. Furthermore, about 1000 germline-expressed genes are lost during DNA elimination, providing an ultimate gene silencing mechanism in the somatic cells. Genomic analysis of the telomere addition sites in an *Ascaris* population revealed a 3-6 kb chromosomal break region (CBR) where telomeres can be added. These CBRs are not associated with motif sequences, common histone marks, or small RNAs, suggesting the break recognition process is sequence-independent. However, all CBRs exhibit more accessible chromatin during DNA elimination, indicating specific mechanisms are involved in this process. To further characterize the DSBs, we used END-seq to identify DNA break sites, end resection, and telomere addition. Using embryos across 11 time points of the 4-8 cell stages, we found that DSBs are introduced before mitosis. Our data also revealed long 3' overhangs are generated after DSB formation, suggesting an extensive end resection process occurs after the initial break. Each breakage site›s resection pattern is unique and may be influenced by the local sequence, nucleosome, and chromatin features. We further observed telomere addition occurs

close to the DNA breakage site. In contrast to the retained ends, we found telomeres rarely heal the eliminated ends of the DNA breaks. We also observed that eliminated ends undergo more resection, suggesting a selective mechanism involved in processing retained vs. eliminated ends. This mechanism is likely associated with the micronuclei formation that engulfs the eliminated DNA, preventing telomere addition. In addition to the 72 CBRs, END-seq revealed 28 alternative break sites within the eliminated DNA. These break sites may provide a fail-safe mechanism to ensure PDE occurs. Overall, our results provide insights into the DNA break and telomere healing processes and lead to new questions in *Ascaris* PDE.

1693F **Understanding the function of the C-terminus of Mms21 in genome stability** Yee Mon Thu[1], Nkechinye Baadi[2], Samuel Li[1] [1]Colby College, [2]Biology, Colby College

MMS21 and its human homolog NSMCE2 have various functions in DNA damage repair and response pathways, which play a vital role in defense against genetic diseases and disorders. Mechanistically, Mms21 functions as an E3 SUMO (small-ubiquitin like modifier) ligase which conjugates SUMO peptides to target proteins with the help of E1 and E2 SUMO ligases. We generated mms21Δ22 mutant in S. cerevisiae, a mutant in which the C-terminus is absent, to approximately mimic the human mutation found in a rare genetic disorder. We observed that a mutant of RAD5 (key regulator of the damage tolerance pathway) and the mms21Δ22 mutant exhibit a negative genetic interaction in the presence of methyl methanesulfonate (MMS), a DNA alkylating agent. This data suggests that Rad5 and the pathway regulated by the C-terminus of Mms21 operate in parallel to counteract MMS-induced DNA damage. Rad5 can function as an E3 ubiquitin ligase to initiate the template switching pathway. In addition, the helicase activity of Rad5 modulates fork regression when the replication fork encounters impediments. To further understand this intricate and essential network of DNA damage response and repair pathways, we will determine if the mms21Δ22 mutant genetically interacts with the rad5 helicase mutant or the rad5 E3 ubiquitin ligase mutant. In addition, we are performing an experiment to understand if the human MMS21 gene, NSMCE2, can functionally rescue the lack of yeast MMS21 gene using the plasmid shuffling assay. In this assay, wild type cells are first transformed with MMS21 on a high copy plasmid. This allows us to knock out the endogenous MMS21, which is essential for cellular survival. Cells with the episomal copy of MMS21 are then transformed with the plasmid carrying NSMCE2. Resulting cells with episomal copies of MMS21 and NSMCE2 will then be grown in the presence of a toxicant (5-FOA) that would force cells to lose the plasmid containing MMS21, while retaining the plasmid with NSMCE2. Growing mms21Δ cells with the episomal copy of MMS21 in 5-FOA led to lethality, confirming the feasibility of the approach. We are currently generating all appropriate strains to determine if the human copy can functionally complement the yeast gene. If such a system is successful, we will characterize cellular and molecular significance of additional NSMCE2 mutations associated with human genetic conditions using this approach.

1694F **The BUDdy System: investigation of GEF and GTPase function in the mitotic exit network in*S. cerevisiae*** Nathalie Merisca, Jennifer Madu, Daniel Sullivan, Anupama Seshan Biology, Emmanuel College

The mitotic exit network, or MEN, in budding yeast, is a Ras-like signaling pathway that governs the cell cycle transition from the end of mitosis into G1. MEN activation is coupled to proper chromosome segregation, and more specifically to the entry of the daughter spindle pole body (dSPB) into the bud. The GTPase Tem1 acts at the top of the pathway and is activated in late anaphase. Tem1 is localized to SPBs and SPB localization is required for its activity. The kinase Kin4 inhibits MEN by preventing Tem1 from localizing. The protein Lte1 inhibits Kin4 and is localized to the daughter cell cortex from S phase to late anaphase. Lte1 prevents Kin4 from binding to the dSPB and in this manner acts as an indirect activator of Tem1. However, Lte1 contains N-terminal and C-terminal guanine nucleotide exchange factor (GEF) domains. These domains are important for Lte1 localization, but we hypothesized that these GEF-homology domains are also important for Lte1 to directly activate Tem1. In this study, we analyzed the impacts of amino acid substitutions within predicted Helix B of Lte1's C-terminal GEF homology domain, which we hypothesized to be important for Lte1 function based on homology modeling with two known GEFs, Sos and RasGRF1. We found that the majority of substitutions (20 out of 25) produced stable protein. We also found that the localization pattern of the Helix B mutants corresponded with their functionality. However, one mutant, I694A, was well localized but still unable to promote mitotic exit. Two other mutants, I683A and N687T, were localized normally, but are only partially functional in promoting mitotic exit. These are the first alleles of *LTE1* where localization does not correlate with functionality. We further investigate how these alleles of *LTE1* impact the localization and activation of Tem1. Our research indicates that budding yeast can be a useful model to study signaling pathway paradigms.

1695F **Genetic interaction studies to understand the function of the C-terminus of Mms21 in genome stability** Cheung Li[1], Lindsey Kim[1], Aleksandra Avramenko[1], Yee Mon Thu[2] [1]Colby College, [2]Biology, Colby College

Sumoylation of proteins coordinates various cellular mechanisms responsible for ensuring genome integrity. Sumoylation is carried out by E1, E2 and E3 SUMO ligases, which facilitate the covalent linkage of small ubiquitin-like modifiers (SUMO) peptide to target proteins. The importance of sumoylation in genome integrity is underscored by genetic disorders associated

with mutations in the SUMO pathway. Truncation mutants of Mms21, an E3 SUMO ligase, have been observed in a rare genetic condition. To characterize how one of these mutations may affect the function of Mms21, we generated the mutant in *Saccharomyces cerevisiae*. This mutant, *mms21Δ22*, lacks the last 22 amino acids of the protein but does not affect the residues in the E3 ligase domain. *mms21Δ22* mutants exhibit negative genetic interaction with *rad5* mutants, suggesting that the C-terminus of Mms21 regulates DNA damage response pathways acting in parallel with the template switching pathway. However, *mms21Δ22* mutants show little or no genetic interaction with *slx4* or *mms4* mutants. These data imply that processes modulated by the Mms21 C-terminus work in tandem with DNA repair pathways that utilize structure specific endonucleases. We found that the SUMO-interacting motif within the C-terminus is not responsible for the phenotype observed. Since the error-prone translesion synthesis functions in parallel to the template switching pathway, we are examining the mutagenic potential of *mms21Δ22* mutants in response to DNA damaging agents. In addition, we are currently performing a dosage suppression screen to uncover which genes cooperate with the C-terminal regulation of Mms21.

1696F **Determining the role of lysine residues in the regulation and function of the DNA mismatch repair protein Msh6.** Kalila Daveron[1], Kayla Carmichael[2], Joanna Haye-Bertolozzi[2] [1]Biology, Xavier University of Louisiana, [2]Xavier University of Louisiana

The DNA Mismatch Repair (MMR) system functions to protect the integrity of the genome in living organisms. Defects in the DNA MMR process can result in increased mutation rates, which in humans can result in Lynch syndrome and increased cancer susceptibility. The initial step of MMR, mismatch recognition, is performed by the protein complex MutSα (Msh2/Msh6). Previous research showed that absence of the lysine acetyl transferase General Control Nonderepressible (Gcn5) results in the increased stability of Msh2. It is also known that Gcn5 acetylates certain lysine residues in Msh6. Additionally, absence of the ubiquitin ligase Modulator of Transcription (Not4) resulted in increased Msh2 protein levels. Our aim is to establish the role of Gcn5 in the regulation of MutSα. We hypothesized that lysine residues in Msh6 may be acetylated by Gcn5, stabilizing the complex. To test our hypothesis we mutated lysine residues, potential Gcn5 acetylation sites, in Msh6 singly and in combination. Lysine residues targeted were primarily in the N-terminus of Msh6 and were changed to alanine, arginine, or glutamine. Using the *CAN1* forward mutation assay, the Msh6 lysine mutants were tested for impact on MMR function in the yeast *Saccharomyces cerevisiae*. Initial analysis of lysine residues shows varying effects on MMR in the mutants tested. We have identified other lysine residues in Msh6 that will be altered to test the effect of mutagenesis on Msh6 protein function.

1697F **Investigating the origin and nature of half-crossover cascades in *Saccharomyces cerevisiae*** Camryn Schmelzer, Ruth Watson, Juan Lucas Argueso Environmental and Radiological Health Sciences, Colorado State University

A half-crossover (HC) cascade is a mutagenic sub-pathway of homologous recombination (HR), where a singular DNA double-stranded break (DSB) is able to trigger the formation of multiple chromosomal translocations. When a DSB is formed but only one of the two broken ends is captured to engage in HR, the repair will proceed through Break-Induced Replication (BIR). In BIR, the single-ended DSB invades a homologous donor sequence and uses it to initiate DNA replication that extends through the end of the template chromosome. Failure of BIR initiation and/or irregular processing of BIR intermediates can lead to half-crossover (HC) formation, in which the single-ended DSB is healed through a translocation, breaking the donor molecule in the process, leaving it with only one DNA end to engage in HR repair. This futile repair attempt may spawn another half-crossover, thus resulting the “cascading” nature of the mechanism. HC cascades can lead to cycles of translocations until BIR occurs to end the series of rearrangements. Structure Selective Endonucleases (SSEs) have been implicated in HC formation. Generally, SSEs cut residual DNA joint molecules, such as D-loops and Holliday Junctions, to conclude HR repair. However, the premature activation of SSEs, such as Mus81 and Yen1, during BIR may generate deleterious HCs. Additionally, Pol32 is an accessory subunit of Poldand largely required for BIR; cells deficient for Pol32 are likely predisposed to HC cascade formation. We have constructed an experimental system in *Saccharomyces cerevisiae* designed to phenotypically identify clones that specifically experienced a cascade of HCs followed by one BIR event that heals the chromosome. We are investigating the roles of Mus81, Yen1, and Pol32 in HC cascade formation, measuring the frequency of HC cascades, and characterizing the resultant rearrangements through Pulse Field Gel Electrophoresis (PFGE) and targeted long-read sequencing across translocation junctions. We postulate HC cascades result from an imbalance between BIR initiation and SSE activation.

1698F **CRISPR is a source of genotoxic stress, even in the absence of gRNA** Stephan C Baehr[1], Samuel Perez[1], Adalyn Brown[1], Alyssa Cenzano[1,2], Katelyn Hancock[1], Lea Patrick[1], Michael Lynch[1] [1]Biodesign Institute, Mechanisms of Evolution, Arizona State University, [2]ASU Preparatory Academy Polytechnic High School

CRISPR is a gene editing platform with seemingly endless applications, from engineering to science and medicine. The excitement of CRISPR’s potential has lead to hundreds of clinical trials aimed at treating human genetic diseases. However, the field is aware that CRISPR’s promise is not entirely without risk; CRISPR by its nature modifies the genome, and may have off-target effects. No enzyme or machine is perfect. Researchers have diligently sought to quantify mutation risk, by testing a specific locus, or set of loci. However, to date, we are unaware of any experiment using the most sensitive detection methods

of evolutionary biology, which can detect error rates that may occur one in a trillion bases per cell division. We demonstrate a mutational hazard of expressing Cas9 to an untargeted locus of yeast, *S. cerevisiae*, which demonstrates a dose-response relationship with increasing Cas9 expression. We find a modest 1.2-fold to 2.7-fold increase in mutation rate, as quantified by fluctuation test. We demonstrate that gRNA appears to increase the mutagenicity of CRISPR, be it Cas9, Cas14, or an Adenine Base Editor (ABE). We note that if indels are the primary means of mutation mechanism in Cas9, the indel rate may be 10-fold higher in our high-expression yeast lines relative to WT controls.

1699F **Checking Set1 activity to ensure meiotic progression** Jayne E Steitz[1], Yasmin Mourad[1], Alexa Sirchio[2], Michael Law[1] [1]Biology, Stockton University, [2]Biological Sciences, Fordham University

Cell fate determination requires faithfully integrating external environmental signaling cues with internal genetic and epigenetic status. Mistakes in signal interpretation can lead to developmental defects or cancer. While the genetics of dividing and differentiating cells are identical, the epigenetics of the cells must be reprogrammed to accommodate distinct fates. In diploid cells of the budding yeast Saccharomyces cerevisiae, mitosis is stimulated in rich growth conditions while meiosis is induced during nutrient depletion. In yeast, many epigenetic regulators are dispensable for mitosis but are essential for meiosis, highlighting the importance of reprogramming the epigenome during this process. Meiosis has several distinct events including pre-meiotic S phase, homologous pairing and recombination, and two rounds of cell division which yield four haploid gametes. Prior to these events, several checkpoint pathways are in place to ensure mistakes are not made prior to cell division. Using a genetic approach, recent work from our laboratory demonstrated that the histone H3 Lys4 methyltransferase, SET1, is essential for progression through homologous recombination and meiosis I, suggesting a key role in satisfying the checkpoints that survey these processes. Surprisingly, Western blot analyses indicate that Set1 is degraded during meiosis, which contradicts its essential behavior for meiosis I. To determine the requirement of Set1 for advancement through meiotic checkpoints, we utilized chemical treatments that induce checkpoint arrest and measured myc-Set1 levels by Western blotting analyses. Yeast harboring myc-Set1 were induced to enter meiosis and treated with 1) hydroxyurea (HU) or 2) nocodazole. HU treatment causes DNA damage, which will arrest cells prior to pre-meiotic S-phase. In contrast, nocodazole results in tubulin depolymerization and will induce the spindle assembly checkpoint prior to meiosis I. We predict Set1 degradation should be initiated following its supportive role in executing the important early meiotic hallmarks of homologous recombination and meiosis I. These experiments will pinpoint when Set1 activity is necessary for cells to efficiently execute the meiotic program.

1700F **Investigating the Role of Mismatch Repair in Promoting Trinucleotide Repeat Expansions in *Saccharomyces cerevisiae*** Katherine M Casazza, Gregory Williams, Lauren Johengen, Maxwell Keller, Brett Irwin, Samantha Phelps, Natalie Lamb, Jennifer A Surtees Biochemistry, University at Buffalo

Mismatch repair (MMR) is an important DNA repair mechanism that protects genomic stability through correcting polymerase errors at the replication fork. In most eukaryotes, including *Saccharomyces cerevisiae* and humans, MMR is initiated by MutS homolog (MSH) complexes, Msh2-Msh3 and Msh2-Msh6, recognizing and binding misincorporations and insertion/deletion loops, respectively. MSH-DNA complexes recruit and activate the latent endonuclease activity of downstream MutL homolog (MLH) complexes, Mlh1-Pms1 and/or Mlh1-Mlh3, to nick the DNA and recruit downstream repair factors. A third MLH complex, Mlh1-Mlh2, lacks endonuclease activity and acts as an accessory factor for both Msh2-Msh3- and Msh2-Msh6-mediated MMR. Deficiencies in the MMR system can lead to Lynch syndrome, a predisposition to cancer and microsatellite instability. While MMR is critical for protecting the genome by preventing mutagenesis, components of the MMR system have been implicated in promoting trinucleotide repeat (TNR) expansions. TNR expansions are the cause of over 40 neurodegenerative and neuromuscular diseases such as Huntington's disease and myotonic dystrophy which are caused by *CAG* and *CTG* repeats respectively. In stark contrast to their function in promoting replication fidelity, components of the MMR system exhibit a pathogenic role through promoting TNR expansions. Using an *in vivo* TNR assay to measure expansion frequencies in *S. cerevisiae*, we previously demonstrated that Msh2-Msh3 can promote genome instability through both *CAG* and *CTG* expansions *in vivo*. This is consistent with work in mice and humans as well as GWAS studies that identified Msh3 as a genetic modifier of expansions. Our work aims to elucidate mechanistic details of Msh3 promoted expansions of threshold length TNR tracts and their distinctions from Msh3-mediated repair. We have demonstrated that Msh3 specific DNA binding is not sufficient for TNR instability. Additionally, we highlight the involvement of all three MLH complexes in promoting tract expansions *in vivo*. Our data demonstrate the importance of an active, albeit aberrant, MMR pathway in promoting TNR expansions. We also report distinct MSH-DNA binding between MMR and TNR structures, *in vitro*. We propose a model in which the TNR DNA structure directs allosteric changes within Msh2-Msh3 that promote a pathogenic Msh2-Msh3-mediated MMR that ultimately leads to TNR expansions.

1701F **Size *Does* Matter: The characterization of telomere function in germ cell development in *Danio rerio*** Jessica MacNeil[1], Sophie Chaviari[1], Cheyenne Petrino[2], Amelia Babb[2], Kellee Siegfried[2] [1]Biology, University of Massachusetts, Boston, [2]University of Massachusetts, Boston

Infertility is a growing problem worldwide and according to the WHO, 48 million couples across the globe suffer from it, so many couples turn to Assisted Reproductive Technology (ART) to become pregnant. A main readout of successful ART can be telomere integrity because telomeres are necessary in aiding the movement of chromosomes during meiosis so that the segregation of chromosomes happens correctly. Telomeres are well known for their role in aging; however, they play an important and understudied role during meiosis. In meiosis, telomeres attach to the nuclear envelope (NE) via a protein chain spanning the NE that interacts with cytoplasmic motor proteins. Through this interaction, chromosomes are shuffled within the nucleus to facilitate homologous chromosome pairing. If this process does not proceed normally, aneuploid gametes may be produced leading to infertility or congenital defects in offspring. Through studying the necessity of telomere length using *telomerase reverse transcriptase* (*tert*) mutant zebrafish, I aim to tease apart the functions of telomeres during gamete development. The *tert* mutant phenotype includes decreased telomere length, aberrant germ cell development, and infertility. I hypothesize that the gonad defects are in part due to problems in meiosis resulting from the shortened telomeres. I found that the meiotic cells in the testis are more sensitive to telomere shortening than non-meiotic cells. By analyzing germ cells undergoing meiosis at 5 months of age, I found an increase of spermatocytes in the leptotene stage of meiotic prophase I, which is when telomeres attach to the NE, suggesting that telomeres of appropriate length are required for meiotic progression. I also found there to be an increase in yH2ax staining in mutant pachytene nuclei, suggesting problems in resolving DSBs necessary for recombination. To get a complete picture of how meiosis is disrupted by shortened telomeres, I am assaying for telomere-NE attachment, homologous chromosome pairing and synapsis, and recombination in fish of different ages. Overall, I will learn how different aspects of meiosis can be affected by shortened telomeres and the importance that telomeres have not only on aging, but in meiosis and overall fertility.

1702S **Formation and stabilization of crossover compartments during *C. elegans* meiosis** Celja Uebel[1], Anne Villeneuve[2] [1]Developmental Biology, Stanford University, [2]Developmental Biology and Genetics, Stanford University

Crossovers (COs) are required to ensure proper segregation of homologous chromosomes during meiosis I in most eukaryotes, yet CO formation is a tightly restricted process. Many organisms designate only a single CO site per chromosome pair or per chromosome arm, despite a substantial excess of the double-strand DNA breaks (DSBs) that serve as the initiating events of recombination, with the majority of DSBs being repaired as non-COs. This restriction of CO formation necessitates that each site designated to become a CO must reliably mature as a CO. Prior research led to a working model in which: 1) A distinct architecture of recombination factors and the formation of a spatially-separated CO-specific repair compartment protects and stabilizes CO intermediates, and 2) Positive feedback involving CDK-2/COSA-1-mediated phosphorylation and scaffold-like properties of the C-terminal tail of MutSγ component MSH-5 combine to promote full recruitment and activity of CO-promoting complexes within the CO-specific repair compartment, thereby conferring robustness to CO designation and maturation. We will expand on this model through our analysis of COSA-2 (CO-Site Associated-2), a new factor identified in an updated genetic screen for mutants defective in meiotic chromosome segregation.

COSA-2 is essential for CO formation, and it becomes abruptly localized to six bright foci per nucleus at late pachynema, colocalizing with other recombination factors at the single CO-designated site per chromosome pair. Moreover, COSA-2 is not required for loading of recombination factors during earlier stages of meiotic prophase, but is specifically required for retention/accumulation of pro-CO factors at late-pachytene CO sites. Super-resolution microscopy reveals a dynamic sub-localization of COSA-2 within CO-site compartments relative to the positions of factors previously inferred to interact with underlying recombination intermediates. The COSA-2 protein contains charged patches and is intrinsically disordered, hallmarks of hub proteins with the potential to bind multiple partners or act as central scaffolds for large multi-protein assemblages. Together these features suggest that COSA-2 may function as “molecular glue”, serving as a hub protein to organize/stabilize the spatially separated compartments that promote CO factor accumulation and architecture of CO-designated sites, thus ensuring formation of a single CO event per chromosome pair.

1703S **Comparison of meiotic proteins REC-1/HIM-5 in *Caenorhabditis elegans* and *Caenorhabditis briggsae*** Michelle Scuzzarella, Judy Yanowitz MWRI, Magee-Womens Research Institute

DNA double-strand breaks (DSBs) are highly deleterious, yet necessary for exchange of genetic material during crossover (CO) formation in meiosis. CO formation is required for the physical connection and proper alignment of homologs to prevent nondisjunction of chromosomes during meiosis I.

In *C. elegans,* most chromosomes receive a single CO on the recombinogenic and less gene-dense chromosome arms, while avoiding the more gene rich chromosome centers. In this organism, the *rec-1* gene is responsible for maintaining normal CO distribution. The number of CO events remains unchanged in *rec-1*mutants, while the location of crossovers is altered. The *him-5* gene plays a role in maintaining normal crossover distribution, while also promoting DSB formation on the X chromosome. When both genes are mutated, more severe defects in DSB formation are observed with nondisjunction of

autosomes also occurring.

Prior studies showed that the *rec-1* and *him-5* genes are distantly related paralogs with a single ancestral gene in other *Caenorhabditis* species. The gene coding for REC-1 is in a synteny block on LG I that is conserved in order and orientation in at least six other *Caenorhabditis* species but the genes in the *rec-1* position show greater sequence similarity with *C. elegans him-5*, which is found on LG V. This evidence, as well as their redundant roles in meiosis suggest that *rec-1* and *him-5* are distantly related paralogs.

We have used the CRISPR/Cas9 gene editing system to create a knockout mutant in the *Caenorhabditis briggsae* paralog of these two genes, *cbg25171*, with the intention of exploring the evolution of these genes and the effect on meiosis in a closely related species. The presence of males in this population has been observed, as well as an increased number of univalents at diakinesis, which indicates an increase in nondisjunction. Based on preliminary data, we hypothesize that *cbg25171* plays a similar role in *C. briggsae*meiosis as both *rec-1* and *him-5* do together in *C. elegans* despite low sequence similarity. Further functional analysis of the *C. briggsae* mutations of the *rec-1/him-5* paralog will be presented.

1704S **Characterization of mutations that affect sex-specific thermotolerance of the synaptonemal complex in *C. elegans* germ cells** Nicolas Lee, Cori Cahoon, Diana Libuda University of Oregon

Meiosis is a specialized form of cell division carried out by sexually reproducing organisms to produce haploid gametes, such as sperm and eggs. Various facets of meiosis are sexually dimorphic which impacts how each sex responds to environmental stressors, such as temperature. Unlike oogenesis, spermatogenesis is extremely sensitive to changes in temperature with heat exposure strongly linked to male infertility, increased DNA damage, and cancer. Although the mechanisms behind this heat-induced male infertility are not clearly defined, our recent data suggests that sexual dimorphisms in the synaptonemal complex (SC), a meiosis-specific chromosome structure, may contribute to the heat sensitivity of spermatocytes in *Caenorhabditis elegans*. The SC is essential for fertility in both sexes, but is highly sensitive to acute heat exposure only in developing sperm. Heat stress of the SC in spermatocytes prompts early SC disassembly and the shortening of the *C. elegans* germline. To determine the proteins that render the SC temperature sensitive, specifically in sperm, we performed a fluorescent-based forward genetic screen. The ongoing genetic screen has identified 26 mutants that alter the SC response to heat by enhancing or suppressing the heat induced SC defects, or displaying other sperm-specific SC defects such as a complete loss of the SC. To identify the causal mutations behind each mutant phenotype, we are utilizing whole genome sequencing and SNP-based recombination mapping. From this analysis, we are generating a candidate list of genes from the genomic region linked to the causal mutation. With established computation pipelines, we are characterizing the candidate genes to uncover the proteins contributing to the temperature sensitivity of sperm. Overall, these studies will reveal the molecular mechanisms by which each mutant alters SC heat sensitivity providing insights into the processes causing heat-induced male infertility.

1705S **The sex specific function of CLS-2 during male sperm meiosis in *Caenorhabditis elegans*** Allen M Ramsey, Sebastian Gomez, Cuc Huynh, Vanessa Cota San Francisco State University

Roughly 1 in 6 people will struggle with infertility, with male factors contributing to 50% of all cases. We know that anucleate or aneuploid sperm can fertilize an oocyte leading to chromosomal abnormalities in embryos. Utilizing Caenorhabditis elegans (*C. elegans*) as a model organism, we aim to elucidate the mechanism of chromosome segregation during sperm meiosis.

The machinery driving faithful chromosome segregation is integral in avoiding chromosomal abnormalities. The central spindle and centrosomes are the primary structures that drive the movement of chromosomes during cell division. Sperm only have centrosomes, unlike oocytes and mitotic cells. The CLS-2 kinetochore protein plays a key role in mediating the attachment of centrosomes to chromosomes. In *C. elegans*, we see a sperm specific localization of CLS-2 surrounding the chromosomes, a distinct difference from other types of cell division. In both mitosis and oocyte meiosis CLS-2 generates the central spindle while localized to the midzone and dissipates as soon as the central spindle has formed, whereas we see CLS-2 stay localized to the chromosomes throughout the entire meiotic process in sperm. We hypothesize that chromosome segregation in sperm meiosis is driven by the centrosomes and that CLS-2 mediates centrosome association of sister chromatids during bi-orientation at metaphase II. The change in localization and persistence of the CLS-2 supports a more integral chromosome-associated function during sperm meiosis. The primary ability of CLS-2 as a microtubule stabilizer suggests that throughout the two segregations seen in sperm meiosis there is a more dynamic machinery involved compared to mitosis and oocyte meiosis.

To test this, we will utilize 3-D reconstruction of live imaged *C. elegans* with three endogenous fluorescent reporters allowing for the visualization of CLS-2's localization in relation to chromosomes and microtubules. We expect to see a similar velocity between centrosome and chromosome dynamics supporting centrosome driven segregation. We will also compare chromosome and centrosome dynamics alongside CLS-2 localization with that of a CLS-2 protein depleted worms utilizing the Auxin Induced Degron Pathway. This will allow for clear characterization of deleterious phenotypes with the expectation of

failed chromosome biorientation and a longer time between each phase of meiosis when CLS-2 is depleted.

1706S **Investigating B chromosome dynamics during female meiosis in *Drosophila melanogaster*** Mengjia Lin[1,2], Stacey Hanlon[1,2] [1]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

B chromosomes, which are extra, non-essential chromosomes, were recently discovered in a single mutant laboratory stock of *D. melanogaster.* This mutant background promotes meiotic drive of the B chromosomes in females, resulting in their biased transmission to progeny. The arrangement of B chromosomes at the metaphase I arrest is abnormal and is associated with elevated B chromosome transmission, but it is unknown if the B chromosomes also display abnormal behavior prior to this arrest (in prometaphase I) or after the arrest during the meiotic divisions. Using fluorescent *in situ* hybridization (FISH) and immunofluorescence (IF) to visualize the B chromosomes and the meiotic spindle, respectively, we first examined the arrangement of B chromosomes in prometaphase I when chromosomes are forming attachments to the meiotic spindle and are highly dynamic. We aim to present our findings that will show where the B chromosomes are before settling into a single DNA mass at the metaphase I arrest. We have currently shifted our investigation to visualizing B chromosome dynamics during the meiotic divisions by collecting freshly laid eggs that are in late-stage meiosis since the meiotic divisions rapidly occur as the egg is moving through the oviduct. Our goal is to observe the location of the B chromosomes at each division stage and elucidate how they are taking advantage of the asymmetry of female meiosis to promote their own transmission. Understanding B chromosome dynamics during female meiosis will provide novel insights into how non-essential, newly formed chromosomes can be transmitted with bias, promoting their maintenance in a population over many generations.

1707S **Maintenance of genomic integrity in the male germline of *Drosophila melanogaster*** Kate Lemons, Kent Golic University of Utah

The germline is integral to survival of a species. To maintain genome integrity in the germline there must be effective and rigorous systems to eliminate cells with DNA damage, such as unrepaired double strand breaks (DSBs). The DNA Damage Response has been well studied and defined in somatic cells: at its core, the protein kinase Chk2 phosphorylates the transcription factor P53 ultimately leading to apoptosis of the cell. Previous research has shown that, in the germline, Chk2 is necessary to eliminate cells with DSBs but P53 is not. We further examined the role of Chk2 in maintaining germline integrity. We found that each of Chk2's functional domains are required for its function. Critically, the necessity of the kinase domain indicates that it acts by phosphorylating a downstream target. However, since P53 is not involved in eliminating cells with damaged DNA in the germline, there must be an alternate target. In some systems Chk2 has been shown to phosphorylate and inactivate String (Stg, aka Cdc25), a positive regulator of cell cycle progression. We found that overexpression of stg^{+} mimics the phenotype of *chk2-null*. This strongly suggests that Chk2 phosphorylates Stg to halt cell cycle progression, and that this is the mechanism used to eliminate germline cells with unrepaired DNA damage.

1708S **Mechanism of natural variation in double-strand break repair:** Shahrzad Hajiarbabi, Erin Kelleher Biology and Biochemistry, University of Houston

DNA repair is critical to maintaining genome sequence fidelity in the face of endogenous and environmental mutagens, yet individuals in natural populations often differ in their capacity to repair different forms of DNA damage. We previously mapped natural genetic variation in wild-type *Drosophila melanogaster* in sensitivity to double-stranded breaks (DSBs) to a large locus near the 2nd chromosome centromere. Mutagen-sensitive flies are extremely sensitive to DSB caused by X-ray irradiation and *P*-element transposition, however, the DNA damage response or repair pathways whose reduced function explains these mutagen-sensitive phenotypes remains unknown. Persistent DSBs will lead to the activation of kinases which are responsible for the phosphorylation of downstream genes involved in the induction of cell cycle arrest, repair, and eventually apoptosis. DSBs in *D. melanogaster* are preferentially repaired through homologous recombination repair (HR); however, canonical non-homologous end joining (c-NHEJ) and alternative end joining (alt-EJ) pathways are also used to repair DSBs. To uncover the mechanism of mutagen sensitivity, I am comparing mutagen-sensitive and tolerant alleles with respect to 1) sensitivity to a panel of mutagens that lead to DSBs and 2) repair products of DSBs. Unique sensitivity to particular classes of mutagens can be diagnostic of specific repair deficiencies. Similarly, differences in repair products can be diagnostic of repair processes whose function is reduced.

We discovered that sensitive alleles exhibit dominant and strong sensitivity to Nitrogen mustard, which causes interstrand crosslinks, as well as weaker recessive sensitivity to Hydroxyurea. In contrast, sensitive alleles do not differ from wild-type flies in their response to mutagens such as Camptothecin, Etoposide, and Methyl methanesulfonate. While interstrand crosslink repair remains poorly understood, evidence suggests it requires homologous recombination. Future examination of repair products will reveal any differences in homologous recombination between mutagen-sensitive and tolerant flies. Together, my experiments will point to why individuals differ with respect to sensitivity to DNA damage.

1709S **In *Drosophila melanogaster*, survivors of Blm-deficient development exhibit a neurodegenerative phenotype that includes sleep and circadian rhythm disruption** Tesla Presnell, Ava Hasenoehrl, Brayden Graves, Abigail Brown, Sara Hathaway, Jayden Youngren, Eric Stoffregen Lewis-Clark State College

DNA damage caused by a lack of maternally loaded Blm protein during early embryonic development in *Drosophila melanogaster* results in significant embryonic lethality. It is unknown, however, how this DNA damage affects normal physiologic processes in the few surviving individuals. We investigated whether this developmental abnormality (Blm-deficiency induced DNA damage) causes neurologic dysfunction in adult survivors. We hypothesized that DNA damage exposure in early development would cause increased neurodegeneration and disrupt normal circadian and sleep patterns. Circadian rhythms are the internal body clocks that cycle approximately every 24 hours and regulate many daily behaviors and physiological processes, including sleep. To test our hypotheses, we collected adult progeny from Blm^{-} mothers crossed to Blm^{+} fathers and from the reciprocal cross, Blm^{+} mothers crossed to Blm^{-} fathers. In both crosses, the progeny were heterozygous for *Blm*, but one set developed with maternally loaded Blm protein and one without. We used a climbing assay to measure motor function and saw a significant decrease in climbing ability at multiple ages in adult flies that developed without maternal Blm, suggesting increased neurodegeneration. We investigated sleep and circadian rhythms using a continuous activity monitor. Our data suggests abnormal circadian patterns and disrupted sleep in flies that developed without maternal Blm protein. Each of these phenotypes has been associated with aging in flies. However, we did not detect an increase in protein aggregates in flies that developed without maternal Blm, suggesting our phenotypes are not a result of accelerated aging in these flies.

1710S **Maternal effect of RNASEH2C loss in *Drosophila melanogaster* during embryogenesis** Sara K Martin[1], Mitch McVey[2] [1]Tufts University, [2]Biology Department, Tufts University

RNASE H2 is a three subunit enzyme that resolves RNA-DNA hybrids and cleaves at incorporated ribonucleotides (rNMPs) in the genome to maintain genome integrity. In mammals these functions are vital to DNA replication and knockout mice for the RNASE H2 are embryonic lethal. Hypomorphic mutations in the three subunits cause an autoinflammatory disease in humans Aicardi-Goutières syndrome. While RNASEH2A is the catalytic subunit, loss of the accessory subunits B or C also prevents catalytic activity.

In order to study the functions of RNASE H2 in an in vivo model, I made *Rnaseh2c* knockout mutants in *Drosophila melanogaster*. Unlike their murine counterparts, homozygous *Rnaseh2c* mutant flies were viable providing an opportunity to study loss of RNASEH2C in an in vivo model. Maternal transcripts are used in the first two hours of rapid replication during Drosophila embryogenesis raising the question if maternal loading from heterozygous mothers enabled homozygous *Rnaseh2c* null fly embryos to survive.

When homozygous *Rnaseh2c* females were crossed to wild-type males the hatching rate was significantly reduced compared to wild-type females mated to wild-type males (59% relative to 91%). Observationally very few heterozygous larvae from homozygous mothers survived to adult hood. On the other hand, heterozygous *Rnaseh2c* embryos from wild-type mothers had a normal hatching rate and most larvae survived to adulthood. This implies that loss of RNASEH2C during the first few hours of embryogenesis impairs development in heterozygous progeny. In a startling contrast, homozygous embryos derived from homozygous mothers and fathers had a normal hatching rate and no observed increased larval death.

One untested hypothesis to explain this unexpected result is that loss of RNASEH2C during the first two hours of embryogenesis results in accumulation of RNA-DNA hybrids and rNMPs that Drosophila embryos can tolerate, but activation of the zygotic genome brings back RNASE H2 activity that acts on accumulated substrates and results in toxic intermediates. Currently, we are measuring levels of RNA-DNA hybrids and rNMPs during embryogenesis, and monitoring markers of DNA damage across embryogenesis to assess how they correlate with activation of the zygotic genome.

1711S ***Y*-chromosomes create a survival disadvantage in male *Drosophila melanogaster* that lack Blm protein during early development** Jayden Youngren, Abigail Brown, Brayden Graves, Ava Hasenoehrl, Sara Hathaway, Tesla Presnell, Eric Stoffregen Lewis-Clark State College

The absence of maternally provided Blm DNA helicase during early embryonic development leads to severe DNA damage in *Drosophila melanogaster*. This Blm-deficiency is lethal to most embryos. An extreme sex bias is seen in the small percent that do survive, where ~70-90% of the population is female. This female sex-bias amongst the surviving progeny correlates with repetitive DNA content. The female karyotype (*XX*) contains less repetitive DNA compared to the male karyotype (*XY*), due to the highly repetitive nature of the *Y* chromosome in *Drosophila*. Additionally, embryos from Blm^{-} mothers have a higher probability of aneuploid sex chromosome karyotypes caused by increased meiotic nondisjunction. These karyotypes

include *XO*, where no second sex chromosome exists and results in a male phenotype, and *XXY*, where an extra *X* chromosome results in a female phenotype. We hypothesized that, due to differences in repetitive DNA content, *XO* males would be more common than *XXY* females amongst the survivors of Blm-deficient early development. Additionally, we hypothesized that *XO* males would exhibit longer lifespans than *XY* males because of the reduced repetitive DNA content present during Blm-deficient development. To test these hypotheses, we assayed for the presence or absence of a *Y* chromosome in male and female survivors of Blm-deficient development (from Blm^{-} mothers) and also compared the karyotypes of adult male flies (*XO* vs. *XY*) that exhibited shorter vs. longer lifespans. As expected, we saw significantly more *XO* than *XXY* adult progeny survive Blm-deficient development and *XO* males from Blm^{-} mothers outlive *XY* males.

1712S **Metabolic dysfunction in survivors of Blm-deficient development in *Drosophila melanogaster*** Sara Hathaway, Abigail Brown, Ava Hasenoehrl, Brayden Graves, Tesla Presnell, Jayden Youngren, Eric Stoffregen Lewis-Clark State College

Blm DNA helicase is essential for proper DNA replication during early development in *Drosophila melanogaster*. Blm^{-} mothers, who do not provision their eggs with functional Blm protein, exhibit a maternal-effect lethality. Nearly all progeny from Blm^{-} mothers die prior to larval hatching; however, a few progeny do survive to adulthood (<10% of embryos). These survivors provide a model in which to study the effects of DNA damage during early development on healthspan. We hypothesized that survivors of Blm-deficient development would display alterations in metabolic function. We tested whether development without Blm protein affected triglyceride, free glucose, or total glucose levels in adult flies. Our data suggests that Blm-deficient development results in lower triglyceride levels in adult flies (male and female) and in reduced glycogen levels in adult males. We saw no significant differences in free glucose levels (males and females) or in glycogen levels in females that developed with or without Blm. These data suggest that the DNA damage sustained by embryos lacking Blm during early cell cycles affects metabolic processes, particularly those related to nutrient storage, into adulthood.

1713S **Physiological Response of DGRP lines to hypoxia and cold stress.** Amelia E May[1], Natalia Rivera Rincon[1], Emma Saurette[1], Ulku Huma Altindag[1], Laurie Stevison[1,2] [1]Biological Sciences, Auburn University, [2]Auburn University

A major consequence of global climate change is the exposure to extreme fluctuations in temperature, forcing populations to shift in their ranges. One expected result would be the shift in the geographic range of species to occupy novel niches such as high-altitude environments. Since these environments are typically also quite cold, we examined the interaction between low oxygen and temperatures on fruit fly physiology. Based on previous work on the DGRP related to chill coma and response to oxidative stress, we selected five stocks, three mutually sensitive and two mutually tolerant to these two stressors. We crossed these stocks to a common tester stock with quadruple X-linked recessive visible mutant markers to assay changes in recombination. We then placed crosses in four possible treatment conditions throughout development – (1) control at 25°C and 21% Oxygen, (2) Low Temperature at 18°C and 21% Oxygen, (3) Low Oxygen at 25°C and 8% Oxygen, and (4) Combined at 18°C and 8% Oxygen. Oxygen concentration was controlled with the use of custom hypoxia chambers from Biospherix and certified pre-mixed tanks from AirGas. Here, I focus on the physiological results from these four treatment groups. Specifically, we measured individual changes for females and males in weight (N=377), oxygen consumption (N=284), carbon dioxide emission (N=284), and critical thermal maximum (N=283) across the five strains and four treatment groups. Our weight results showed a significant difference due to the interaction of sex, strain, and treatments. Using both oxygen and carbon dioxide exchange, we were able to measure respiratory quotient (RQ). Finally, our CTmax results showed a decrease in thermal tolerance in sensitive strains when exposed to low temperatures and hypoxia, while tolerant strains exhibited the highest values of CTmax across treatments and strains. Phenotypic attributes across four genetic loci were collected in D. melanogaster for the recombination data. While our analysis of this work is ongoing, it will inform our understanding of how various genetic backgrounds can influence response to climate change.

1714S **Delayed lagging strand synthesis drives asymmetric histone incorporation and promotes progenitor cell reprogramming in the *Drosophila* male germline** Brendon E M Davis[1], Jonathan Snedeker[1], Rajesh Ranjan[1,2], Matthew Wooten[1,3], Vikrant Mahajan[1], Xin Chen[1,2] [1]Biology, Johns Hopkins University, [2]Biology, Howard Hughes Medical Institute, [3]Fred Hutchinson Cancer Research Center

In the *Drosophila* male germline lineage, stem cells display asymmetric histone inheritance while progenitor germ cells exhibit an overall symmetric pattern. We report that an essential molecular mechanism underlying this cellular specificity is delayed lagging strand synthesis, which drives old histone incorporation into the leading strand and is found to be sufficient to generate asymmetric histone incorporation in non-stem cells. A candidate screen identified that proteins involved in lagging-strand synthesis, such as DNA Polymerase α (Polα) and DNA Polymerase δ (Polδ), are expressed at reduced levels in stem cells compared to non-stem cells in the same germline lineage. Genetically compromising Polα induces the replication-coupled histone incorporation pattern in non-stem cells to be indistinguishable from that in stem cells, and this is recapitulated using a Polα inhibitor in a concentration-dependent manner. Furthermore, stem cell-derived chromatin fibers display a temporal difference in the replication of both DNA strands and show a significantly higher degree of old histone recycling to the

leading strand than in progenitor cell-derived fibers. However, upon reducing Polα levels in non-stem cells, the progenitor cell chromatin fibers display asymmetric old histone recycling just like stem cell-derived fibers. Importantly, these altered chromatin features in progenitor cells allow them to act like *bona fide* stem cells under both pathological and physiological conditions. Together, these results indicate that developmentally programmed expression of key DNA replication components could be manipulated to promote progenitor cell dedifferentiation and redifferentiation, suggesting a new pathway for cell reprogramming in stem cell lineages.

1715S **Initiation Mechanism of Drosophila Interchromosome Effect** Bowen Man[1], Bowen Man[2] [1]Case Western Reserve University, [2]Biology, Case Western Reserve University

In Drosophila crossover formation during meiosis, a heterozygous inversion on a homologous chromosome pair will lead to decrease of crossover formation on the local chromosome pair, but the number of crossovers would increase on other chromosomes. This phenotype is caused by a check point that induce prolonged prophase that allows more crossover formation. In this presentation, I would test two different models, axial integrity and lacking crossovers on local homologous chromosomes, that could explain the initiation mechanism of this check point.

1716S **Cell competition and the fate of segmental aneuploidies of varying size** Nicholas Baker, Jacky Chuen Genetics, Albert Einstein College of Medicine

Aneuploidy, the gain or loss of chromosomes, causes of birth defects and miscarriage. Almost all tumors are aneuploid, as are senescent cells, and aneuploidy promotes both tumorigenesis and premature aging. Sporadic aneuploid cells are often lost from otherwise normal tissues. There are many unresolved questions about the mechanisms of these effects.

The distribution of genes encoding 80 ribosomal protein genes across the chromosomes means that in ribosome biogenesis is usually affected in aneuploid cells. Genetic screens in fruitflies identified a transcriptional stress response to ribosomal protein gene haploinsufficiency, mediated by the bZip protein Xrp1. Overall reductions in cellular and organismal growth rates, and in global translation rate, are among the transcriptional effects of Xrp1 expression. An Impaired Ribosome Biogenesis Checkpoint (IRBC) is seen in vertebrates, where it is mediated by p53.

Individual cells carrying Rp gene mutations experience Xrp1-dependent cell competition, ie they are actively eliminated in the presence of wild type cells. FLP/FRT-mediated excision of chromosome previously showed that cell competition based on Rp gene dose is responsible for removing sporadic aneuploid cells (PMID33847264).

Monosomies lacking *Rp* genes typically extend over only ≤2% of the genome. Now we have investigated the effects of more extensive chromosome loss. Heterozygous deletion of larger and larger chromosome regions increasingly reduced cell viability, leading to less and less growth, until eventually cells with heterozygous deletion of sufficient material no longer survive, even without cell competition. Beyond even this point, FLP recombination of FRT sites separated by much of a chromosome arm can produce hyperplasia and tumors. These data suggest that segmental monosomy can create three kinds of stress according to the extent of sequence lost: the first and most sensitive eliminates aneuploid cells through cell competition due to Rp gene haploinsufficiency, mediated by a specific transcriptional stress response; the second is a less sensitive, cell-autonomous toxicity, that becomes significant when larger portions of the genome are affected; finally, still more extensive aneuploidy seems to promote hyperplasia. Taken together, this data suggests that, whereas most aneuploidies, or segmental monosomies at least, inhibit cellular growth through cell-autonomous and non-autonomous pathways, particular, highly aneuploid genotypes exist that promote hyperplasia and tumorigenesis.

1717S **Pumilio-dependent stabilization of Nipped-B mRNA during meiotic prophase is required for cohesion rejuvenation in *Drosophila* oocytes** Muhammad Abdul Haseeb[1], Erin Dickert[2], Sharon Bickel[2] [1]Biological Sciences, Dartmouth College, [2]Dartmouth College

Accurate chromosome segregation of homologs during the first meiotic division requires that cohesion along the arms of sister chromatids be maintained from S phase until anaphase I, when release of arm cohesion allows recombinant homologs to segregate to opposite poles. In Drosophila oocytes, a cohesion rejuvenation program operates during meiotic prophase to promote *de novo* loading of cohesin and formation of new cohesive linkages after cohesion is originally established during premeiotic DNA replication. Disruption of rejuvenation causes premature loss of arm cohesion and missegregation of recombinant homologs. To identify proteins required for cohesion rejuvenation but not S phase establishment, we performed an RNAi screen and found that knockdown of the RNA-binding protein Pumilio during meiotic prophase causes a significant increase in meiotic chromosome segregation errors. Interestingly, Gerber *et al.* (2006) have reported that immunoprecipitation of Pumilio protein from Drosophila ovary extracts pulls down transcripts encoding the cohesin loader, Nipped-B. Using FISH to

detect premature loss of meiotic cohesion in mature Drosophila oocytes, we discovered that Pumilio, like Nipped-B, is essential for maintaining arm cohesion during prophase. We have developed an Smc1 “tag-switch” reporter that expresses GFP-tagged Smc1 exclusively during meiotic prophase and allows us to directly visualize association of newly synthesized cohesin with oocyte chromosomes after premeiotic S phase. Using this tool, we have found that when Nipped-B is knocked down during meiotic prophase, cohesin loading on the arms of meiotic chromosomes is severely disrupted. We observe a similar phenotype in Pumilio knockdown oocytes. Moreover, RNA-seq analysis using ovary extracts from control and Pumilio knockdown extracts revealed a significant reduction in the abundance of Nipped-B transcripts when Pumilio is knocked down during meiotic prophase. Collectively, our findings suggest that cohesion defects in Pumilio knockdown oocytes arise from the destabilization of Nipped-B transcripts and subsequent disruption of Nipped-B dependent cohesin loading and rejuvenation during meiotic prophase. Experiments are currently underway to visualize and quantify Nipped-B protein on meiotic chromosomes in order to test the hypothesis that Pumilio knockdown during meiotic prophase causes a reduction of chromosome associated Nipped-B protein in Drosophila oocytes.

1718S **Topoisomerase 3b Facilitates piRNA Biogenesis to Promote Transposon Silencing and Germ Cell Development** weiping shen[1], Seung kyu Lee[1], William wen[1], yuyoung Joo[2,3], yutong xue[1], shuaikun su[1], tiangyi zhang[4], megan zhang[5], Jinshui Fan[1], yongqing zhang[1], supriyo de[1], alexei sharov[1], Manolis Maragkakis[1], weidong Wang[1] [1]NIA, [2]NIH, [3]niaaa, [4]tianyi.zhang@nih.gov, [5]Yale university

Topoisomerases typically function in the nucleus to relieve topological stress in DNA. Here we show that a DNA-RNA dual-activity topoisomerase complex, Top3b-TDRD3, primarily localized in the cytoplasm of Drosophila germ cells, biochemically and/or genetically interacts with piRNA processing enzymes to promote piRNA biogenesis, post-transcriptional and cotranscriptional silencing of transposons, and germ cell development. A Top3b point mutant that lacks topoisomerase activity is defective in silencing of a transposon reporter, suggesting that Top3b may relieve topological stress in RNA. Top3b-TDRD3 null mutants in flies exhibit phenotypes similar to those of piRNA pathway mutants; including reduced fertility. Notably, mutations in Top3b or TDRD3 are associated with fertility disorders in both humans and mice. Our data reveal a novel role of topoisomerase in RNA-based processes—piRNA biogenesis and transposon silencing; and suggest a possible mechanism of how Top3b-TDRD3 mutations can lead to germ cell dysfunction across vertebrates and invertebrates.

1719S **Regulation of mammalian meiosis by the SUMO-conjugating enzyme UBC9** Sunkyung Lee[1], Dhananjaya Kulkarni[1,2], Yungdae Ryu[1], Tianhong Lu[1], Neil Hunter[1,2] [1]Microbiology and Molecular Genetics, University of California Davis, [2]Howard Hughes Medical Institute, University of California Davis

During meiotic prophase I, the homolog axis organizes chromosome structure and is required for several aspects of homologous recombination including the formation of DNA double-strand breaks (DSB), template selection (homolog versus sister chromatid), and crossing over. Our recent studies have identified the small ubiquitin-like modifier (SUMO) as a key regulator of meiotic prophase I[1)] and in budding yeast, all known components of the homolog axes were identified as targets[2)]. However, targets in mammals and how SUMO regulates axis function remain unknown.

We identified human SYCP3 as a direct (E3-ligase independent) target of the E2-conjugase UBC9 *in vitro*. Although SYCP3 is SUMOylated at multiple sites, modification is largely dependent on a single consensus binding motif for UBC9. Typically, direct targets of UBC9 require additional binding interfaces to stabilize interaction and enable efficient modification. We found that auto-SUMOylation of UBC9 at K14 is important for SYCP3 modification suggesting a bipartite interaction comprising: (i) binding of the SUMO consensus motif on SYCP3 by the UBC9 catalytic cleft; (ii) binding of SUMO conjugated to UBC9-K14 by SYCP3.

To understand the *in vivo* significance of UBC9-K14 SUMOylation for meiosis, we generated a knock-in mouse line Ubc9K14R/K14R. While Ubc9K14R/K14R spermatocytes have ostensibly normal chromosome synapsis, preliminary analysis indicates that chromosome axes are shorter and crossing over is reduced. Ongoing experiments are exploring the fertility of Ubc9K14R/K14R mice, the composition of chromosome axes, and how meiotic recombination is altered. This analysis will provide important insights into how SUMO modification regulates meiosis in mammals.

[References]

1. Rao HB, Qiao H, Bhatt SK, Bailey LR, Tran HD, Bourne SL, Qiu W, Deshpande A, Sharma AN, Beebout CJ, Pezza RJ, Hunter N. A SUMO-ubiquitin relay recruits proteasomes to chromosome axes to regulate meiotic recombination. Science. 2017 Jan 27;355(6323):403-407. doi: 10.1126/science.aaf6407. Epub 2017 Jan 5. PMID: 28059716; PMCID: PMC5569317.

2. Bhagwat NR, Owens SN, Ito M, Boinapalli JV, Poa P, Ditzel A, Kopparapu S, Mahalawat M, Davies OR, Collins SR, Johnson JR, Krogan NJ, Hunter N. SUMO is a pervasive regulator of meiosis. Elife. 2021 Jan 27;10:e57720. doi: 10.7554/eLife.57720. PMCID: PMC7924959.

1720S **Relationships Between Germline Mutation Rates and Reproductive Success** Alexis Garretson[1,2], Beth L Dumont[1,2] [1]Genetics, The Jackson Laboratory, [2]Tufts University

The mutation rate is a critical genomic parameter, as germline mutations are the cause of heritable genetic disorders and the ultimate source of evolutionary novelty. The germline mutation rate is a complex trait, but we know little about how it is modified by heritable genetic features. In humans, elevated mutational burdens have been tentatively linked to reproductive capacity. Higher mutation rates are associated with lower reproductive success and earlier age at last birth. However, the fertility-associated consequences of natural variation in mutation rates are incompletely understood, mainly due to the absence of mutation rate and reproductive phenotype estimates across large population cohorts. Here we harness the breeding funnel design of the Collaborative Cross (CC) mouse population alongside dense breeding records from each CC strain to profile the accumulation of mutations over ~30 inbreeding generations and to evaluate associations between mutation rate and multiple metrics of reproductive fitness. Using a pan-genome, graph-based variant caller applied to publicly available whole-genome sequences for 69 CC strains, we identify ~11,000 *de novo* mutations (DNMs) specific to each CC line and absent from the eight founder strains, including many DNMs with projected functional effects. The per generation rate of mutation accumulation varies 11-fold among strains, exceeding null expectations and pointing to the presence of mutation rate modifiers segregating among the 8 CC founder strains. Mutation rates are negatively correlated with several metrics of reproductive capacity, including average litter size, interbirth interval, and age at the first litter. Further, strains with higher mutation rates exhibit a more rapid increase in the interbirth interval and concomitant decreases in litter size with age. Our findings suggest that germline mutation rates in mice are associated with multiple metrics of fertility and reproductive aging, revealing shared genetic mechanisms underpinning variation in these complex traits.

1721S **Mechanisms and fitness consequences of human embryonic aneuploidy inferred from 129,479 blastocyst-stage embryos** Sara A Carioscia[1], Arjun Biddanda[2], Ivan Vogel[3], Eva Hoffman[3], Rajiv C McCoy[2] [1]Johns Hopkins University, [2]Biology, Johns Hopkins University, [3]University of Copenhagen

Less than half of all human conceptions survive to live birth. Extra or missing chromosomes (aneuploidies) are the primary cause of pregnancy losses, many of which occur during the preimplantation stages. Data from preimplantation genetic testing for aneuploidy (PGT-A) from trophectoderm biopsies of blastocyst-stage in vitro fertilized (IVF) embryos offers a unique view into the origins of aneuploidy and the landscape of dosage sensitivity during the first five days after fertilization. Here we report detailed patterns of chromosome abnormalities detected in biopsies of 129,479 blastocyst-stage IVF embryos from 20,900 sets of patients. Using genome-wide array-based genotyping of embryo biopsies, alongside parental genotypes, we infer transmission of individual parental homologs and assign parental origins of aneuploidies. The scale of the data offers unique insight into the origins of rare abnormalities in genome-wide ploidy. We then leverage this data to investigate biases in transmission of different parental haplotypes among the IVF embryos.

We find that both single-chromosome gains and losses as well as whole-genome gains and losses primarily involve maternal chromosomes. Maternal meiotic-origin trisomies were strongly enriched on chromosomes 15, 16, 21, and 22, consistent with previous findings. Because the predominant mechanisms of maternal meiotic error produce equal ratios of monosomies and trisomies (via reciprocal gain and loss in the egg and polar body, or vice versa), imbalance in this ratio offers insight into impact of selection of gains versus losses during cleavage-stage embryonic development. Moreover, we are able to understand the proportions of different error types (meiosis I, meiosis II, mitosis) across age tranches. This temporal information allows us to characterize the landscape of aneuploidy-generating mechanisms and their relative contributions in early human development, building a foundation to investigate the genetic basis of aneuploidy phenotypes.

1722S **Investigating the Role of DNA Secondary Structures in Centromere Specification** Pei Shyuen Ooi, Jitendra Thakur Biology, Emory University

Centromeres are chromosomal locations that are essential for proper chromosome segregation to avoid aneuploidy and genomic instability, both of which are hallmarks of many cancers and genetic disorders. Centromeres are specified by the strong enrichment of a specialized epigenetic chromatin, where histone H3 is replaced by its variant called centromere protein-A (CENP-A). How CENP-A is targeted to centromere locations remains a longstanding question. Recent studies have shown that centromeres are enriched for DNA secondary structures that differ from the Watson-Crick double helix model of DNA. These studies suggest that these DNA secondary structures may contribute to centromere specification by targeting CENP-A to centromeric DNA. I aim to investigate the function of DNA secondary structures in specifying centromere locations by identifying types of DNA structures and their protein partners that may be involved in CENP-A targeting. To do so, I am using the latest high-throughput secondary structure mapping techniques to identify the sites and types of DNA structures at centromeres. Additionally, I am also using CRISPR/Cas9-based perturbations of potential centromeric protein partners of DNA secondary structures. Interestingly, I have found that a specific type of DNA secondary structure has pockets of

depletion within centromeric regions but is abundant at centromere flanking regions. The findings indicate that this class of DNA secondary structures is processed rapidly by their protein partners at these pockets of depletion, suggesting a potential mechanism for CENP-A targeting. This research will reveal high-resolution footprints of DNA secondary structures at centromeres. Additionally, this research will identify DNA secondary structure protein partners, uncovering the importance of potential interactions of DNA secondary structures with centromeric proteins in centromere specification.

1723S **Characterizing Mutagenesis Across Developmental Time with sciATAC-seq** Yu-Chen Pan, David Mas Ponte, Diego Calderon, Annabel Beichman, Kelley Harris University of Washington

Although somatic mutations are most often studied in the context of cancer and tumor development, they occur spontaneously throughout an individual's lifetime. Recently, there has been a growing interest in studying somatic mutations in healthy tissues to better understand mutational processes in normal cells and to trace cell lineages during development. However, somatic mutations tend to be found in small clusters of cells, and extra-sensitive technologies are needed to detect them. Existing methods for identifying somatic mutations include single clone cell culture expansion, organoid culture and laser capture dissection, but they are relatively low throughput and expensive. Given these limitations, we developed a novel approach to detect somatic mutations with single-cell indexing (sci) ATAC-seq data, which is more scalable and already abundantly available for reanalysis. By calling mutations found in reads derived from chromatin-accessible regions, we detected putative somatic and germline mutations and explored the mutagenesis landscape across tissues during development using previously published sci-ATAC-seq data from fruit fly embryos and human fetal samples. In the fruit fly embryonic development analysis, each 1-mer mutation type showed a slight increase in mutational load over developmental time, affecting both the soma and the germline. The 3-mer mutation spectrum appeared consistent with previous mutation accumulation experiments. We also performed mutational signature analysis across multiple human fetal tissues, which was able to distinguish between somatic and germline mutations and reveal differentiation across tissue types. In our work, we demonstrated that we can successfully detect and differentiate somatic and germline mutations with our pipeline. This new approach will allow us to reuse available datasets to study somatic mutagenesis at a single cell resolution for a relatively affordable cost, enhancing scalability and opening up the possibility for using spontaneous somatic mutations to trace cell lineages.

1724S **Genetic interactions between fission yeast Cdc24 and PCNA** Sally G Pasion[1], Ntsaum S Vang[2] [1]Biology, San Francisco State University, [2]Ross University School of Medicine

In the fission yeast, *Schizosaccharomyces pombe*, Cdc24 has been implicated in lagging strand synthesis and DNA repair (Gould et al., 1998; Tanaka et al., 1999, Zhang et al., 2016). Although Cdc24 is a novel replication/repair protein, it has physical and genetic interactions with the DNA replication and repair protein, Proliferating Cell Nuclear Antigen, PCNA. PCNA has been shown to interact with Cdc24 when the proteins are ectopically expressed in yeast cells (Tanaka *et al*., 1999), and has been shown to be a multicopy suppressor of Cdc24 ts lethality (Reynolds et al., 1998). It is not clear which PCNA components are required for these interactions. In this study, we constructed *cdc24-ts pcn1.K164A* double mutants and report that they exhibited a synthetic growth defect. We also determined that *pcn1.K164A* is epistatic to *cdc24-M81*, in response to UV irradiation, suggesting that Cdc24 and PCNA both function in the same DNA repair pathway. Further, we identified that the PCNA K164A mutation and mutations along the PCNA interdomain-connecting loop (IDCL) affect the ability of PCNA to suppress *cdc24* temperature sensitivity.

1725S **Structural and biochemical basis of retrotransposition by human LINE-1** Akanksha Thawani[1], Eva Nogales[2], Kathy Collins[3] [1]Molecular and Cell Biology, UC Berkeley, [2]UC Berkeley, HHMI, [3]UC Berkeley

The Long Interspersed Element-1 (L1) retrotransposon has generated nearly one-third of the human genome and serves as an active source of genetic diversity and human disease. L1 spreads via a mechanism termed target-primed reverse transcription, in which the encoded enzyme (ORF2 protein, or ORF2p) nicks the target DNA to prime reverse transcription of its own or non-self RNAs. Here, we purified the full-length LINE-1 ORF2p and biochemically reconstituted robust target-primed reverse transcription with template RNA and target site DNA. We report cryo-electron microscopy structures of the human L1ORF2p bound to structured template RNAs and initiating cDNA synthesis. The template polyadenosine tract is recognized in a sequence-specific manner by five distinct domains. Among them, a novel RNA-binding domain bends the template backbone to allow engagement of an RNA stem with the LINE-1 ORF2p C-terminal segment. In addition, our structure and biochemical reconstitutions demonstrate a surprising requirement for the target DNA architecture for nicking and target-primed reverse transcription. By synthesizing biochemical and structural data, our work provides key insights into the mechanism of ongoing transposition in the human genome and informs the engineering of retrotransposon proteins for gene therapy.

1726S **Rethinking Mutation Accumulation: Measuring Mutation Bursts in *Saccharomyces cerevisiae*** Joseph A Stewart[1], Mackenzie Wienke[2], Camryn Schmelzer[1], Lucas Argueso[1] [1]Environmental and Radiological Health Sciences, Colorado State

University, [2]Biology, Colorado State University

In the field of evolution, gradualism is the process of incremental adaptation supported by a slow and random accumulation of mutations that, over time, lead to genetic diversification and fitness gains. Although this Darwinian model is well supported and widely accepted, it cannot always explain the rapid changes seen in some instances such as tumors with extremely high and complex mutation loads. Recent reports in various organisms, including from our group using *Saccharomyces cerevisiae*, provide evidence for an additional mode of rapid and non-independent accumulation of chromosomal rearrangements. We have used a yeast model to follow the accumulation of structural genomic rearrangements such as loss of heterozygosity (LOH). We found that while chances of a single LOH event happening are very low, two or more LOH tracts co-occurred at rates 14- to 200-fold higher than expected if these events were independent of each other; therefore, the conventional process of slow and independent accumulation of mutations are not sufficient to account for every change in the genome. In the present study, we focused on temporal kinetics of bursts of LOH accumulation in yeast. We developed a hybrid diploid yeast experimental strain that enables identification of LOH event both through counter-selection and visual screening for colony color. This hybrid strain, made from the S288c and SK1 genetic backgrounds, possesses ~55,000 heterozygous SNPs distributed throughout the genome and allows for ease of tracking LOH events through sequencing. The screening approach was used in combination with microcultures (one cell grown for 5 or 6 divisions) in phylogenetic analyses that unambiguously revealed cases where multiple LOH events co-occurred in the same cell division cycle. Collectively, these studies offer support for punctuated bursts of mutation accumulation caused by systemic genomic instability (SGI). Additionally, we have investigated a potential mechanism that influences SGI, namely global noise in gene expression.

1727S **Lack of Ung1 increases *S. cerevisiae* sensitivity to anticancer ruthenium complex KP1019** Rishi Narayan, Andrew Chinn, Pamela Hanson Furman University

Although platinum-based drugs are often used to treat cancer, they have limitations that include severe side effects, as well as development of drug resistance. Some ruthenium-based complexes overcome these limitations as they cause fewer side effects while remaining effective against multidrug resistant cancer and yeast cells. One such ruthenium-based drug is indazolium trans-[tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019). In a phase I clinical trial, KP1019 had low toxicity yet stabilized disease progression in 5 of 6 evaluable patients. In addition to poorly characterized cytoplasmic targets, DNA appears to be an important target of KP1019 in both yeast and cancer cells, as previous studies show the drug can cause double-strand breaks, DNA adducts, and interstrand crosslinks. An unpublished barcode sequencing screen suggests that yeast lacking the evolutionarily conserved uracil DNA glycosylase Ung1 are hypersensitive to KP1019. Using a growth inhibition assay, we verified this result, determining that the average IC50 value for ung1 deletion strains was significantly lower than wild-type controls (13±5 vs 22±5 ug/mL, respectively; $p<0.05$). This finding suggests that cytosine deamination is an additional type of DNA damage caused by KP1019. Interestingly, acute exposure to 80 ug/mL KP1019 reduced survival rates of wild-type and ung1 mutants to a similar extent with roughly 30% of cells surviving relative to untreated controls. Given the difference in growth inhibition and survival assays, lack of Ung1 appears to have a greater impact on yeast resistance to the cytostatic rather than cytotoxic effects of KP1019. To determine whether loss of Ung1 alters the frequency of KP1019-induced DNA damage, loss of function of the nuclear gene CAN1 was used to measure mutation frequencies. In the absence of drug, ung1 mutants had a 50% higher mutation frequency than wild-type ($p<0.05$). KP1019 increased mutation frequency approximately ten-fold in both strains, but there was not a significant difference between strains. Since Ung1 is localized to both the nucleus and mitochondria in yeast, yet we observed no difference in drug-induced mutation frequency of a nuclear gene, we propose that KP1019 may deaminate mitochondrial DNA, resulting in cytostatic effects. Moreover, since KP1019 has been shown to accumulate in the mitochondria of ovarian cancer cells and Ung1 is involved in mitochondrial DNA repair, the mitochondria is a potential target of KP1019.

1728S **Ccq1 restrains Mre11-mediated degradation to distinguish short telomeres from double-strand breaks** Julien W. Audry[1], Haitao Zhang[1], Carly Kerr[1], Kathleen L Berkner[2], Kurt W Runge[1] [1]Inflammation and Immunity, Cleveland Clinic Lerner Research Institute, [2]Cardiovascular and Metabolic Sciences, Cleveland Clinic Lerner Research Institute

Telomeres cap chromosome ends and are distinguished from DNA double-strand breaks (DSBs) by means of a specialized chromatin composed of DNA repeats bound by a multiprotein complex called shelterin. We investigated the role of shelterin components in distinguishing telomeres from DSBs by studying viable mutants lacking these proteins. Mutants were studied using a *Schizosaccharomyces pombe* model system that induces cutting of a proto-telomere bearing telomere repeats to rapidly form a new stable chromosomal end, in contrast to the rapid degradation seen at a control DSB. Cells lacking the shelterin components Taz1, Rap1, or Poz1 or the telomere-associated protein Rif1 formed a chromosome end that was stable for multiple cell divisions. Surprisingly, cells lacking Ccq1, or impaired for recruiting Ccq1 to the telomere, converted the cleaved proto-telomere to a rapidly degraded DSB. Ccq1 recruits telomerase, establishes heterochromatin and affects DNA damage checkpoint activation; however, these functions were separable from protection of the new telomere by Ccq1.

Telomere degradation in cells lacking Ccq1 was greatly reduced by eliminating nuclease activity of Mre11, a component of the Mre11-Rad50-Nbs1/Xrs2 complex that processes DSBs. Cells lacking Ccq1 and Mre11 that did not degrade the new telomere still activated the cell cycle checkpoint, showing that DNA degradation and checkpoint activation were separable in this mutant. These results demonstrate a novel function for *S. pombe* Ccq1 to effect end-protection by restraining Mre11-dependent degradation of the DNA end.

1729S **Chromosomal Crossing Over During Meiosis: crossover-specific resolution of double Holliday Junctions** John E mccarthy, Neil Hunter UC Davis

Crossing over between homologous chromosomes is required for their accurate segregation during meiosis to produce haploid euploid gametes. A critical step in this process is the formation of double-Holliday Junction (dHJ) recombination intermediates, which are specifically resolved into crossovers products with exchange of chromosome arms, as opposed to a random mixture of crossover and non-crossover products as predicted by canonical models. While the steps leading to dHJ formation are well understood, how dHJs are specifically resolved into crossovers is unclear. We are elucidating the mechanism of dHJ resolution in budding yeast *Saccharomyces cerevisiae*. Recent experiments from our lab evoke a model in which crossover-specific dHJ resolution occurs through a mechanism similar to the initiation steps of DNA mismatch repair. Specifically, sliding clamp PCNA directs the MutLγ endonuclease to create strand-specific nicks on either side of the two Holliday Junctions (HJs). The conserved Bloom helicase/decatenase complex (Sgs1-Top3-Rmi1 in budding yeast) then unwinds the DNA between the nicks to resolve the dHJ. The immediate resolution products are predicted to contain both single-stranded flaps and gaps that require trimming and gap-filling activities to produce mature crossover products. These predictions are being tested *in vivo* using unique molecular-genetics tools developed in budding yeast that enable the synchronization of cells at the dHJ-resolution step, inactivation of pertinent factors in real time, and direct monitoring of dHJ resolution. Our insights will be broadly relevant for understanding meiotic crossing over in higher eukaryotes, including mammals.

1730S **Histone Variant H2A.Z and Linker Histone H1 Influence Chromosome Condensation in *Saccharomyces cerevisiae*** Anna M Rogers, Nola R Neri, Lorencia Chigweshe, Scott Holmes Molecular Biology and Biochemistry, Wesleyan University

Chromosome condensation is essential for the fidelity of chromosome segregation during mitosis and meiosis. Condensation is associated both with local changes in nucleosome structure and larger scale alterations in chromosome topology mediated by the condensin complex. We examined the influence of linker histone H1 and variant histone H2A.Z on chromosome condensation in budding yeast cells. Linker histone H1 has been implicated in local and global compaction of chromatin in multiple eukaryotes, but we observe normal condensation of the rDNA locus in yeast strains lacking H1. However, deletion of the yeast *HTZ1* gene, coding for variant histone H2A.Z, causes a significant defect in rDNA condensation. Loss of H2A.Z does not change condensin association with the rDNA locus, or significantly affect condensin mRNA levels. Prior studies reported that several phenotypes caused by loss of H2A.Z are suppressed by eliminating Swr1, a key component of the SWR complex that deposits H2A.Z in chromatin. We observe that an *htz1Δ swr1Δ* strain has near normal rDNA condensation. Unexpectedly, we find that elimination of the linker histone H1 can also suppress the rDNA condensation defect of *htz1Δ* strains. Our experiments demonstrate that histone H2A.Z promotes chromosome condensation, in part by counteracting activities of histone H1 and the SWR complex.

1731S **Role of telomeric proteins in the repair of a single double-strand break in an expanded CTG trinucleotide repeat** Cécile Palao[1,2], Wilhelm Vaysse-Zinkhöfer[1], Maria Teresa Teixeira[3], Guy-Franck Richard[1] [1]Pasteur Institute, [2]Sorbonne University, [3]Institute of Physico-Chimical Biology

Microsatellites are DNA tandem repeats of 1 to 6 nucleotides. Discovered in the 1960s and considered a long time as “Junk DNA”, microsatellites are known to play many roles in gene regulation and chromosome organization. But they are also involved in some cancers and neurological disorders such as fragile X syndrome, Huntington disease or myotonic dystrophy type 1 (DM1). DM1 is a neurodegenerative disease induced by a pathological expansion of the microsatellite (CTG)n localized in the 3’UTR of the *DMPK* gene. The resulting pathogenic mRNA transcribed from this expanded repeat is responsible for the disease. One possible therapeutic approach would be to reduce the number of CTG trinucleotide repeats below the pathological length in humans, by the use of highly specific DNA endonucleases.

Preliminary results of the feasibility of this approach have been obtained in the model yeast *Saccharomyces cerevisiae*. A double-strand break (DSB) was induced by a TALE nuclease (TALEN) in an expanded CTG trinucleotide repeat integrated in a yeast chromosome. Contraction of the repeat occurs by single-strand annealing (SSA) and leads to reduction of the triplet repeat length. After DSB induction, DNA is resected on both sides of the break, resulting in single-stranded recombinogenic 3’-OH ends, complementary to each other. They can anneal to each other, eventually leading to CTG repeat contraction.

To better understand this mechanism, we studied resection proteins and found that the endonuclease activity of Mre11p as well as its partner Sae2p are necessary to process the DSB within CTG repeats, but not in a non-repeated region. It is known that CTG repeats can form secondary structures *in vitro*, suggesting that Mre11 activity is required to remove these structures that would hinder the loading and processing of DSB ends by other proteins. The deletion of *EXO1*, a gene responsible for late resection steps, showed a minor effect on resection and repair of the same DSB. However, Dna2p, another protein involved in late resection is essential for the processing of the DSB on both ends of the DSB, even in presence of Mre11p and Sae2p.

We also discovered that the deletion of certain telomeric proteins increased resection at a CTG DSB, but not at the same locus that does not contain the repeat tract. The sequence similarity of CTG repeats with telomere sequences in S. cerevisiae (G1-3 T) led us to investigate the potential role of the telomeric machinery in the resection and repair of a DSB induced in a long expanded CTG microsatellite. ChIP experiments were performed to determine whether the telomeric proteins that decrease resection at CTG DSB are enriched at this trinucleotide repeat tract and/or if they are enriched after a DSB within the CTG. RNA-seq experiments were conducted to identify differential gene expression in the presence of CTG repeats in the genome of Saccharomyces cerevisiae. The results obtained will be discussed.

1732V **Investigating the function and regulation of the Haspin homolog, HASP-1, in the *C. elegans* germline** David Wynne University of Portland

The protein kinase Haspin is critical for proper chromosome segregation during cell division. Haspin-dependent phosphorylation of histone H3 recruits the Chromosomal Passenger Complex (CPC) to chromosomes, along with a parallel pathway that depends on the kinase Bub1. Despite long interest in the function of the CPC, Haspin has been studied in relatively few cell types and mostly during mitosis. How the pools of CPC recruited by Haspin and Bub1 are distinct and whether CPC recruitment mechanisms are altered in different cell types and during meiosis remain poorly understood. Recent work in *S. cerevisiae* and mouse oocytes has validated the hypothesis that CPC pools function differently in mitosis and meiosis. We are using *C. elegans* to better understand the mechanisms of Haspin regulation and the function of Haspin-dependent CPC recruitment in the germline. The *C. elegans* gonad contains mitotically dividing nuclei as well as meiotic divisions that produce sperm and oocytes. We previously confirmed that the *C. elegans* Haspin homolog *hasp-1* promotes the mitotic divisions in the germline and spermatogenesis in addition to its demonstrated role in oocyte meiosis. We are generating a series of N-terminal deletion mutations to investigate the mechanisms by which the HASP-1 protein is recruited to chromosomes and activated. These tools will allow us to assess the function of the HASP-1-dependent pool of CPC in the diverse cell types of the *C. elegans* germline.

1733V **RING Finger Proteins in Crossover Designation and Interference** Emerson Frantz, Susan McMahan, Jeff Sekelsky UNC Chapel Hill

The RING Finger proteins Vilya, Narya, and Nenya (VNN) are believed to facilitate crossover designation in meiosis. If crossing over occurs incorrectly, it can result in nondisjunction which can cause genetic conditions like Down Syndrome. VNN are found concentrated in the synaptonemal complex, a structure that forms between chromosomes during meiosis, in locations where crossovers occur. Because of this, we believe that increases in expression of these genes will lead to an increase in crossovers and a decrease in crossover interference. In order to test the role of VNN, a transgene containing the three genes was assembled using a UAS::GAL4 promoter system via Goldenbraid cloning. The completed transgene was injected into Drosophila and paired with a nanos driver to amplify expression. When compared to a negative control, it was found that there are higher rates of crossovers due to higher expression of VNN, indicating that crossover interference also lessens as predicted. Further research to increase our understanding of the genes includes analyzing the VNN transgene with different drivers and creating marked deletions for each individual gene to look at their effects. In order to quantify the dosage of VNN, we also plan on performing qRT-PCR.

1734V **Synaptonemal & CO Analyzer: a novel tool for the analysis of immunofluorescence images in meiotic recombination studies** Elena de la Casa-Esperon[1], Angela Belmonte-Tebar[2], Joaquim Soriano[2] [1]Biochemistry and Molecular Biology, University of Castilla-La Mancha, [2]University of Castilla-La Mancha

Meiotic recombination is fundamental process that not only generates genetic diversity, but is also required for proper chromosome segregation. Chromosomes that do not recombine or have anomalous crossover distributions often separate poorly during the subsequent cell division and end up in abnormal numbers in ova or sperm, causing miscarriage or birth defects. Hence the importance of studying recombination, which in vertebrates is usually performed by immunostaining of chromosome spreads of pachytene-stage spermatocytes or oocytes. This allows to visualize the synaptonemal complex formed between the homologous chromosomes as well as the number and position of the crossovers. But recombination studies often require the analysis of large numbers of cell images, which is very time-consuming and a major bottleneck to achieving

conclusions.

To overcome this limitation, we present a new image analysis tool. Yielding reliable results in half the time of manual analysis, this Fiji/ImageJ macro can analyze microscopy images of many vertebrate species (e.g., mouse, chicken, zebrafish) with diverse antibody labels for crossover and synaptonemal complex studies. Its development under Fiji/ImageJ is specially designed for researchers with different requirements, so they can implement and adapt it to their needs. It is easy-to-use, compatible with different operating systems (Windows and MacOS) and freely accessible through public repositories. In summary, Synaptonemal & CO Analyzer is a free, easy to use, flexible and versatile tool that speeds microscopy image analyses and, hence, facilitates recombination studies.

1735V **Chromatin remodeling complexes function in chromosome segregation and ploidy maintenance** Md Riajul Hossain, Jesus Moreno, Adelle Warford, Ines Pinto Biological Sciences, University of Arkansas

The integrity of genomes requires the faithful segregation of the newly replicated chromosomes during cell division. The main goal of this work is to gain an understanding of the role that chromatin and chromatin remodeling complexes have during mitotic chromosome segregation. To identify proteins that are involved in ploidy maintenance, we carried out a screen of the *S. cerevisiae* non-essential deletion library for genes that when mutated caused ploidy increase. Among the mutants that increased ploidy, we encountered members of the INO80 and SWR1 complex (INO80C and SWR1C). Both ATP-dependent chromatin-remodeling complexes participate in a variety of biological processes including transcription, DNA repair and DNA replication. INO80C catalyzes the eviction of the H2A.Z histone variant replacing it with H2A in nucleosomes. This complex is comprised of 15 subunits, and their specific contribution to chromosome segregations remains largely unknown. The INO80C has been implicated in the maintenance of ploidy through the characterization of mutations of the genes encoding the Ies6 and Ino80 subunits (Chambers et al. doi:10.1101/gad.199976.112), which result in ploidy increase. The SWR1C catalyzes the exchange of H2A for H2A.Z. The yeast SWR1C is comprised of 14 subunits. The Swr1 subunit creates the scaffold of the complex and is essential for its enzymatic activity. To evaluate the contribution of each subunit to chromosome segregation, we tested deletion mutants of all the non-essential subunits of both complexes, and Ts alleles of *ARP4* and *SWC4,* for benomyl sensitivity, ploidy maintenance and chromosome segregation. Furthermore, we analyzed genetic interactions among *ino80Δ* and *swr1Δ,* the catalytic subunits of both complexes. Although both individual mutants increase ploidy, *ino80Δ swr1Δ* double mutant maintains the ploidy increase behavior of *ino80Δ* mutants. This finding suggests that INO80C may have additional functions to ensure proper chromosome segregation, or that there are parallel ways to deposit H2A.Z. Our data indicate that both complexes are required for the maintenance of normal ploidy and genomic stability, but only the catalytic subunits and a few other subunits are required for this function, including the essential ones. Moreover, the Ino80 and Swr1 subunits associate with pericentric chromatin and their presence correlates with the expected H2A.Z turnover.

1736V **Visualization and Immunoprecipitation of distinctively tagged PCH-2 strains in C.elegans** Micaela C Colmenarez, Natalie Gallagher, Valery Ortiz, Dr. Anna Russo, Dr. Needhi Bhalla Molecular, Cell and Developmental Biology, UC Santa Cruz

Mitosis and Meiosis are both essential for proper segregation of chromosomes. Defects in mitosis/meiosis produce aneuploid cells and are associated with cancer progression, birth defects and infertility, underscoring their importance to human health. In this experiment, we used C.elegans to better understand the meiotic and mitotic checkpoint protein, pachytene checkpoint protein (PCH-2). PCH-2's role in preventing aneuploidy is complex in both meiosis and mitosis. Therefore, in order to determine PCH-2's role, we conducted immunoprecipitations on distinctively tagged PCH-2 strains to identify protein interactors and post-translational modifications that may regulate its function.

We will use strains that have PCH-2 tagged at the N or C terminus with Green Fluorescent Protein (GFP) and 3X Flag (3XF) in order to visualize the protein in vivo and immunoprecipitate it. We will also use mutations in PCH-2 that are predicted to bind substrates better (EQ mutants). We have shown that C-terminally tagged PCH-2 is functional during mitosis but not meiosis. I tested whether the N-terminally tagged PCH-2 (3XF::GFP:: PCH2) was functional during meiosis and found that 3XF::GFP::PCH2 exhibited no defects in recombination, similar to control worms and different than pch-2 mutants. We also verified that N-terminally tagged PCH-2 (3XF::GFP::PCH2) was also functional in mitosis. Thus, we have two tagged versions of PCH-2, one functional in both mitosis and meiosis and the other, functional in mitosis. By completing these experiments, we will be able to see if PCH-2 has post-translational modifications or interacts with proteins that specifically affect its function throughout mitosis and meiosis.

1737T **Analyzing the expression of *C. elegans* IGEG-1, a novel EGFR ligand that promotes sleep** Marine Barsegyan, Cheryl Van Buskirk Biology, California State University, Northridge (CSUN)

Sleep is observed across the animal kingdom, both in vertebrates and invertebrates, but its cellular function remains a mystery.

In *C. elegans,* sleep is independent of circadian regulation, providing an ideal model for the study of sleep need independent of day-night timing. Interestingly, these nematodes sleep following exposure to damaging conditions, pointing to cell damage as the molecular basis of sleep need. Evidence of this phenomenon, stress-induced sleep (SIS), has been found in other species, including vertebrates.

In *C. elegans*, SIS is mediated by the activation of Epidermal Growth Factor (EGF) receptors within sleep-promoting neurons. However, the sole previously identified *C. elegans* EGFR ligand, LIN-3, is dispensable for this process. In a genetic screen for SIS-defective mutants, we have identified IGEG-1, a previously uncharacterized EGF family ligand. We have also identified a stress-responsive EGF sheddase, ADM-4, that is required for IGEG-1 processing. Our ADM-4 site of action data suggests that IGEG-1/EGF can be shed from a range of damaged tissues to trigger sleep. To further test this model, we wish to examine the expression pattern of IGEG-1.

As sleep can be induced rapidly following damage, we reason that regulated shedding, rather than *igeg-1* transcription, is the limiting step in this signaling pathway, and we hypothesize that IGEG-1 will be constitutively expressed in a range of tissues. Here, we present insights into the spatial, temporal, and potentially stress-responsive patterns of *igeg-1* gene expression *in vivo* using a Green Fluorescence Protein (GFP) transcriptional reporter, which will inform our understanding of this novel sleep signal.

1738T **Understanding the Activity of Polycystin-2 in Relation to Its Localization in Cilia and Extracellular Vesicles Using C. elegans Model** Carlos Nava Cruz, Juan Wang, Inna A Nikonorova, Elizabeth Desranleau, Jonathan D Walsh, Maureen M Barr Department of Genetics and Human Genetic Institute of New Jersey, Rutgers- The State University of New Jersey

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a form of ciliopathy resulting from mutations in either PKD1 or PKD2. ADPKD is dominantly inherited but thought to be caused by a somatic second hit and loss of heterozygosity. PKD1 and PKD2 encode polycystin-1(PC1) and the TRP channel polycystin-2(PC2). The localization of these polycystins to cilia and extracellular vesicles (EVs) is conserved across species, ranging from the nematode C. elegans to humans. The relationship between the function of polycystins and their localization to cilia and EVs, as well as the dynamic interplay between ciliary and EV localization in contributing to ADPKD, remains unclear. In this study, we will utilize C. elegans model to investigate the cellular localization of PKD-2 in both gain-of-function and pathogenic loss-of-function contexts, with the aim of elucidating how PKD-2 activity is related to its ciliary and EV localization.
Constitutively active PC2(F604P) more effectively rescues cyst formation in the pronephric kidney when endogenous PC2 expression is down-regulated (Pavel et al 2016). The F604 residue in PC2 is highly conserved and corresponds to F445 in C. elegans PKD-2. We have generated an endogenous PKD-2::GFP CRISPR reporter and will engineer this F445P gain-of-function mutation into the PKD-2::GFP locus. This approach will enable us to directly observe the ciliary and extracellular vesicle (EV) localization of PKD-2(F445P) and assess its effects on male mating behavior, which is a read-out of polycystin ciliary function. Employing the same methodology, we will introduce other pathogenic mutations into PC2 that inhibit its channel activity, and monitor their impacts on PKD-2 expression, trafficking, localization, and function. Investigating these PC2 mutations in C. elegans will provide insights into the partitioning of PC2 between cilia and EVs. These studies may elucidate how modulating the interplay between ciliary and EV localization of polycystins could attenuate or prevent aberrant cilia and EV signaling, ultimately reducing cyst development in ADPKD patients.

REFERNCE:

Pavel *et al*, 2016, Function and regulation of TRPP2 ion channel revealed by a gain-of-function mutant. Proc Natl Acad Sci U S A. 113: E2363-72. PMID: 27071085 Wang *et al*,2021, Sensory cilia act as a specialized venue for regulated extracellular vesicle biogenesis and signaling. Curr Biol. ;31:3943-3951. PMID: 34270950

1739T **EFN-4 and VAB-8 act downstream of MAB-5/Hox to promote QL.a and QL.ap posterior migration.** Vedant Jain, Erik Lundquist Molecular Biosciences, University of Kansas

Q neuroblasts are a pair of bilateral neuroblasts that are born in the posterior-lateral region of the animal, with QL on the left and QR on the right. Q cells are the anterior sisters of the V5 seam cells. Initially, QR protrudes and migrates anteriorly over V4 seam cell, whereas QL migrates posteriorly over V5 seam cell. The second phase of migration is Wnt-dependent and begins after the first phase and the first Q cell division. QL descendants QL.a/p encounter EGL-20/Wnt which is a posteriorly expressed Wnt ligand. This ligand leads to the initiation of canonical Wnt pathway and expression of the MAB-5/Hox transcription factor in QL. *mab-5*expression in QL.a enables migration posteriorly over QL.p, after which QL.a undergoes cell division to generate two daughter cells QL.aa and QL.ap. QL.aa undergoes apoptosis, and QL.ap continues migration posteriorly and differentiates into the PQR neuron. *mab-5/Hox* is both necessary and sufficient for posterior migration, as ectopic expression in QR results in posterior migration of QR.ap (AQR neuron). The genes regulated by MAB-5 to drive posterior migration have remained

unknown. Early L1 larval Q cells from *wild-type*, *mab-5* loss-of-function (lof), and *mab-5* gain-of function (gof) strains were FACS sorted and subject to RNA-seq. Differential expression analysis identified genes with increased or decreased expression in these mutants. Expression of the unconventional kinesin gene *vab-8* was reduced in *mab-5* lof and increased in *mab-5* gof. In *vab-8* mutants, QL.a failed to migrate posteriorly from its birthplace, resulting in PQR at the place of QL division. This suggests that *vab-8* is required for posterior QL.a migration. *efn-4/Ephrin* expression was also reduced in *mab-5* lof. In *efn-4* mutants, QL.a undergoes its initial posterior migration, but after division, QL.ap (PQR) fails to complete the final phase of migration, resulting in PQR residing just anterior to the anus. Live imaging suggests that QL.ap differentiates prematurely into the PQR neuron in *efn-4* mutants. These results suggest that *efn-4* affects a later step of QL.ap migration, and that distinct genes might be regulated by *mab-5* to control each step. Transgenic expression of *vab-8* and *efn-4* in the Q cells rescued PQR migration, suggesting that *vab-8* and *efn-4* act autonomously in the Q cells downstream of MAB-5. Through our unique live imaging approach, we have been able to decipher the functional roles of these genes in the posterior migration process. In sum, we have identified two genes, *vab-8* and *efn-4*, that act downstream of *mab-5/Hox*in a transcriptional program to control posterior migration.

1740T **EGL-1 and CED-4, components of the core programmed cell death pathway, inhibit anterior QL.a and QL.p migration, possibly downstream of MAB-5/Hox** Celeste Gormly, Erik Lundquist Molecular Biosciences, University of Kansas

Neuroblasts QL and QR are born bilaterally in the posterior lateral region of the worm between the V4 and V5 hypodermal seam cells. QR migrates anteriorly over V4 and QR migrates posteriorly over V5, and both cells then divide. EGL-20/Wnt is secreted in the posterior region of the worm where QL daughter cells QL.a/p encounter it. EGL-20 drives the expression of MAB-5 in QL via canonical Wnt signaling. MAB-5 is both necessary and sufficient to drive posterior migration. Before, MAB-5 re-programs QL.a to migrate posteriorly, it must prevent anterior migration of QL./p, which is a default state. To identify genes regulated by *mab-5,* we performed FACS sorting and RNA-seq in early L1 Q cells from *mab-5* gain-of-function (gof) and loss-of-function (lof) mutants as well as wild-type worms. Differential expression analysis was used to identify genes that were upregulated and downregulated in each of the mutants compared to the wildtype. Notably, *egl-1* was significantly upregulated in *mab-5* gof. Canonically, EGL-1 activates the programmed cell death pathway (PCD) by inhibiting CED-9/Bcl2, although the PCD pathway is involved in other developmental events such as synapse elimination (Meng et al., 2015). *egl-1* mutations had no effect on posterior QL migration alone, but significantly enhanced anterior QL.ap (PQR) migration defects of a hypomorphic *egl-20* mutants. *ced-4/APAF* also strongly enhanced anterior PQR migration in *egl-20* and *bar-1* hypomorphic mutants. These results suggest that EGL-1 and CED-4 are required to inhibit the anterior migration of QL.a and QL.p, and that EGL-1 might be upregulated by MAB-5 in QL.a/p to inhibit anterior migration. *ced-4* did not suppress *mab-5* gof, consistent with it acting redundantly with other pathways. Future studies are aimed at characterizing other PCD pathway members in this process, as well as determining the mechanisms by which this pathway might inhibit anterior migration, possibly by destabilizing the actin cytoskeleton.

1741T **CED-1 negatively regulates extracellular vesicle release from ciliated sensory neurons into the environment** Tao Ke, Jessica Tanis Department of Biological Sciences, University Of Delware

Extracellular vesicles (EVs) are membrane-wrapped structures containing proteins, RNAs, lipids, and metabolites that are released from most if not all cell types to mediate intercellular communication. The primary cilia of the RnB sensory neurons of male *C. elegans*, which produce different subpopulations of EVs, are surrounded by the ray structural cells (Rnst), RnA dendrites and hypodermal cells. Thus, the number of EVs released into the environment could be determined by both EV shedding from the RnB neurons and the uptake and clearance by Rnst or hypodermal cells. Our previous studies showed that EVs containing the *C. elegans* calcium homeostasis modulator (CALHM) ion channel CLHM-1::tdTomato cargo are shed from the ciliary base, while the EVs with transient receptor potential (TRP) channel PKD-2::GFP cargo primarily bud from the ciliary tip. In the current study, we are investigating whether release of the different EV subpopulations is regulated by *ced-1*, which encodes a transmembrane receptor in engulfing cells that mediates engulfment of apoptotic cell corpses and debris. We hypothesized that CED-1 is important for the uptake and clearance of EVs that are produced at the ciliary base and can be phagocytosed by adjacent cells. Young adult male worms with single-copy insertion of PKD-2::GFP and CLHM-1::tdTomato were imaged with total internal reflection fluorescence (TIRF) microscopy and the number of EVs released into the environment was quantitated with Imaris software. Our imaging data showed that *ced-1(e1735)* worms released 2.0-fold more PKD-2::GFP containing EVs and 2.4-fold more CLHM-1::tdTomato EVs compared to the control. Notably, the relative increase in the number of the EVs with CLHM-1::tdTomato cargo was greater than the EVs with PKD-2::GFP cargo. These data suggest that the CED-1 receptor negatively regulates the number of EVs released into the environment, particularly the EV subpopulation with the CLHM-1::tdTomato cargo that are released from the ciliary base. Further studies to determine the potential roles of CED-1 in the cells surrounding the RnB cilia will delineate whether this receptor is required for EV uptake and has a non-cell-autonomous mechanism to regulate EV release into the environment.

Keywords: *C. elegans*, extracellular vesicles, primary cilia, *ced-1*, CLHM-1, PKD-2

1742T **CASY-1 Orchestrates Foraging Dance: Unveiling the Genetic Symphony of Food-Evoked Locomotion in *C. elegans*** Navneet Shahi, Nisha Kumari, Kavita Babu Centre for Neuroscience, Indian Institute of Science

Food availability in an organism's environment is a critical determinant of its food-seeking and exploration behaviors. The integration of multisensory cues associated with food perception leads to the optimization of locomotion and the development of foraging strategies for survival. Numerous studies emphasize the ecological significance of foraging behavior and the potential physiological factors that influence it. However, a comprehensive understanding of the genetic underpinnings of the foraging behavior regulation remains elusive.

To investigate the intricate neuro-molecular mechanisms responsible for governing the food-responsive behaviors, we have employed the nematode *Caenorhabditis elegans* as a model system. *C. elegans* acts as an ideal model system for this study due to its genetic tractability and the conservation of gene functions with mammalian systems. Several cell-adhesion molecules (CAMs) have been reported to dynamically regulate locomotory circuit function. CASY-1, a mammalian calsyntenin orthologue, is one such CAM predominantly expressed in the sensory neurons and functions to maintain the Cholinergic motor neuron output. Interestingly, our study has found that the *casy-1* mutants display aberrant foraging behavior, characterized by swarming and reduced dispersal to food encounters. Based on these observations, we plan to investigate if *casy-1* present in the sensory neurons can regulate this food-seeking phenotype by modulating the function of the motor neurons.

Notably, Neuromodulatory circuits comprising Neuropeptide receptors (NPR) and their neuropeptide ligands have been reported to regulate the food search and response behaviors in *C. elegans*. Our preliminary experiments have shown that the neuropeptide processing *egl-3* and *egl-21* mutants suppress the foraging defects of *casy-1* mutants. These findings are corroborated by the genetic interactions of *casy-1* with the neuropeptide *flp-21*, and its receptor *npr-1*, in the locomotion assays. This led us to hypothesize that CASY-1 acts through neuropeptidergic signaling to regulate sensory-evoked motor output. This study aims to shed light on the molecular mechanisms through which CASY-1 orchestrates foraging behavior in *C. elegans*, providing valuable insights into the genetic basis of food-related locomotory responses.

1743T **Influence of RasGAP on PXF-1 mediated synaptic development** Reagan Lamb[1], Salvatore Cherra[2] [1]Neuroscience, University of Kentucky, [2]University of Kentucky

Small G proteins coordinate the development of synapses in the nervous system. Pairs of guanine exchange factors (GEFs) and GTPase activating proteins (GAPs) act in a reciprocal nature to modulate the activity of small G proteins. Previously, we found that *pxf-1,* a *Caenorhabditis elegans* RapGEF homolog, promotes synaptic development in cholinergic neurons. Using the cholinergic neuromuscular junction, we sought to identify which GAPs may interact with the PXF-1 pathway to coordinate the development of neuromuscular junctions. We found that a loss of function mutation in *gap-1* restored neuromuscular function to *pxf-1* mutants*.* Using endogenous and transgenic markers of cholinergic synaptic vesicles, we found that *gap-1* mutants also reduced the synaptic vesicle deficits seen in *pxf-1* mutants. To determine whether the canonical target of GAP-1, LET-60/Ras, may be involved in this pathway, we looked at synaptic vesicle phenotypes with a constitutively active mutant, LET-60(G12V). We found that neuronal expression of LET-60(G12V) rescued *pxf-1* mutant synaptic vesicle phenotypes. Since PXF-1 typically activates Rap G protein signaling, we sought to determine whether a Ras GEF may modulate LET-60 signaling and synapse development. We examined whether a putative Ras GEF, *R05G6.10,* displayed any deficits in the development of cholinergic neuromuscular junctions. We found that a deletion in *R05G6.10* decreased synaptic vesicle intensity to similar levels as *pxf-1* mutants. Moreover, double mutants between *R05G6.10* and *pxf-1* did not cause any additional defects in synapse development*.* Overall, these data indicate that LET-60 signaling interacts with PXF-1 to promote the development of neuromuscular junctions. These findings highlight an understudied interaction between G proteins that will contribute to a greater understanding of neuronal developmental.

1744T **Toward understanding the inherent left-right asymmetric differences in *C. elegans* Q neuroblasts** Felipe L. Teixeira, Erik A. Lundquist Molecular Biosciences, University of Kansas

Cell migration is a crucial mechanism for proper development of the nervous system, and the asymmetrical migration of *Caenorhabditis elegans* Q neuroblasts serves as a useful model to study the importance of such mechanism for neuronal development. *C. elegans* Q cells are bilateral neuroblasts born approximately one hour before hatching at similar positions on the left (QL) and right (QR) lateral side of the animal. Despite the position similarity, QL and QR will follow an asymmetrical pattern of migration. During the first four hours after hatching, QL will migrate posteriorly to a position dorsal to the seam cell V5 and QR will migrate anteriorly to a position above V4. After reaching these positions, the Q cells will divide and their descendants migrate further along the anterior-posterior axis. This asymmetry is determined by intrinsic differences between QL and QR during their early migration. To better understand these differences, we are studying the early life of *C. elegans* Q

cells, particularly the period between their birth and first cell division. By using two markers that are co-expressed exclusively on Q cells (*Pegl-17::gfp; Pscm::mCherry*), we were previously able to FACS sort these neuroblasts from arrested L1s to use in bulk RNA-sequencing analysis. To sort younger Q cells, we used microscopy to confirm that these markers are also expressed in embryonic Q cells and we adjusted *C. elegans* growth conditions to stimulate egg holding by gravid adults. This allowed recovery of eggs spanning all stages of embryo development, including eggs in which Q cells were already born. In order to get cells that covered the entirety of QL and QR lifespan, we allowed further embryo development and egg hatching by incubating isolated eggs for three to four hours, which also helped increase the proportion of Q neuroblasts in the cell population. Cells were successfully extracted from the mixed population of eggs and L1s by using a novel combination of chitinase, SDS-DTT and pronase treatment protocols. FACS of dissociated cells allowed for identification and sorting of a cluster of cells that matched *C. elegans* Q neuroblasts. We now plan to use single-cell RNA-sequencing of the sorted Q cell population to identify two expression clusters representing the two Q cells, QL and QR, followed by differential gene expression analysis to identify genes associated with each population.

1745T **The role of peroxisome mediated glial-neuronal communication during Drosophila aging.** Anurag Das, Hua Bai Genetics, Development & cell Biology, Iowa State University

Peroxisomes are responsible for metabolic activities through the regulation of antioxidant enzymes and oxidases. An important process highlighted during cellular aging is when increased oxidative stress leads to a reduced import efficiency of peroxisomal matrix proteins containing a C-terminal peroxisomal targeting signal type 1 (PTS1). One of the key import factors that is predicted to enable cargo proteins holding PTS1 and its delivery to the peroxisomal matrix is the PEX5 (peroxin-5) protein *(Huang et al., 2020)*. Even though there is emerging evidence of peroxisomal distribution in and its dependency on glia cells *(Chung et al., 2020)* but an overarching question remains – "which adult glial subtype among *Drosophila* CNS contains peroxisomes?" We have made a novel identification that the cortex glia has a predominance of peroxisomes in the adult *Drosophila* brain. Further, we aim to establish a closely intertwined connection between peroxisome biology and glial of the adult *Drosophila* during the process of cellular aging. We have seen the dynamic changes in transport of the peroxisomal import machinery from the cytosol to the peroxisome marked with eYFP carrying the peroxisomal targeting signal - PTS1. We demonstrate that eYFP.PTS1 reporter driven by Repo-GAL4, a pan-glial marker, can be used to understand the decline of peroxisomal transport in the glial cells of the adult female *Drosophila* brain. Our preliminary data suggest that peroxisome import declines in aged glial cells. Among future directions, we are investigating how PEX5 impairment in different CNS glial cells, and peroxisome dysfunction affects glial and neuronal function during aging. Thus far, we have seen greater lipid droplet accumulation for cortex glia-PEX5 mutants compared with control as well as other glia drivers combined PEX5 mutants which is supported by literature as previously seen in (*Kis et al., 2015*) where they had shown lipid droplet accumulation being specific to the superficial cortex glial cells in the larval *Drosophila* brain. Our lab generated data also supports a PEX5 dependent decline in the climbing behavior of the flies which supports the idea of peroxisome mediated glia-neuronal cross talk.

1746T **Investigating the roles of *doublesex* and *dissatisfaction* in neurons contributing to female courtship behavior** Kara E Miller, Troy R Shirangi Biology, Villanova University

In *Drosophila*, transcriptional regulators encoded by the *doublesex* (*dsx*) and *dissatisfaction* (*dsf*) genes play important roles in the development of female courtship behaviors, but how they contribute to neuronal sexual development is unclear. Here, we investigated the phenotypic effects from the developmental knockdown of *dsx* and *dsf* transcripts in two female-specific *dsf* and *dsx* coexpressing neurons in the abdominal ganglion, the DDAG_C/D neurons. These neurons contribute to ovipositor extrusion, a courtship rejection behavior performed by mated females, and develop during metamorphosis as remodeled larval neurons. Knockdown of *dsx* in females had no obvious effect upon the DDAG_C/D development, whereas reduction of *dsx* function in males caused the gain of DDAG_C/D neurons. Conversely, knockdown of *dsf* resulted in the loss of the DDAG_C/D neurons in females, while the males were unaffected. Interestingly, optogenetic activation of the DDAG_C/D neurons in males with reduced *dsx* activity induced an extrusion of the terminalia, a behavior resembling ovipositor extrusion of mated females. Taken together, our results suggest that the male isoform of DSX promotes cell death of the DDAG_C/D neurons during metamorphosis, whereas DSF acts as an anti-apoptotic factor for the DDAG_C/D neurons in females. We hypothesize that DSX-M may promote DDAG_C/D cell death by antagonizing DSF's function as a pro-survival factor in the DDAG_C/D neurons.

1747T **Mapping synaptic partners of female-specific neurons that regulate female receptivity in *Drosophila melanogaster*** Micaela Murphy, Troy Shirangi Biology, Villanova University

In *Drosophila*, genes such as *doublesex* (*dsx*), *fruitless* (*fru*), and *dissatisfaction* (*dsf*) identify neurons that regulate sexually dimorphic behaviors in adults. *Dsx*- and *dsf*-expressing interneurons in the abdominal ganglion, named the DDAG neurons, are a small sexually dimorphic population that mediates abdominal behaviors in unmated and mated females that signal

their willingness or unwillingness to mate with courting males. Here, we utilize retro- and *trans*-Tango, two genetic tools for retro- and anterograde transsynaptic tracing, respectively, to identify pre- and postsynaptic partners of the DDAG neurons. Our work in progress has identified a population of interneurons with localized processes in the abdominal ganglion as putative postsynaptic partners of the DDAG neurons in females, mapping the DDAGs and their postsynaptic partners as upstream of abdominal motor neurons for female behavior. Future experiments will test the activity of these putative DDAG postsynaptic neurons for their contribution to courtship behaviors of unmated and mated females. Results from these experiments will provide insight into *dsf* and *dsx* function in the assembly of a sex-specific neuronal circuit that regulates female courtship behavior in *Drosophila*.

1748T **Notch signaling regulates temporal patterning for timely neuroblast elimination in a lineage specific manner** Kendall R Branham, Chhavi Sood, Sarah E Siegrist Biology, University of Virginia

The neurogenic period, where neural stem cells proliferate to produce molecularly distinct progeny in the developing brain, is a critical time of growth in many organisms. Proper brain development is crucial for survival and requires strict regulation of neural stem cell (NSC) divisions along the developmental timeline. In NSCs cell intrinsic programs integrate with extrinsic cues to control periods of rapid growth through temporal patterning genes. Without regulation, NSCs can under proliferate leading to diseases like microcephaly, or over proliferate leading to macrocephaly and tumors. We know programs that control timing of proliferation and elimination of NSCs exist, but many elements of temporal cassettes are still unclear. We currently lack a comprehensive understanding of temporal cassette regulation and how this impacts NSC progeny. To address these questions, we carried out a large-scale RNAi in Drosophila NSCs, known as neuroblasts (NBs), and identified Notch and its ligand, Delta as upstream regulators of temporal cassettes through the early factor Imp. We went on to show that reduced Notch signaling led to persistence of most central brain neuroblasts beyond their normal termination point (Sood et al., 2023). However, in mushroom body neuroblasts (MBNBs) we found loss of Notch resulted in premature elimination. The opposing phenotypes we saw in different NB lineages led us to investigate what factors could confer these differences. We studied the MBNB specific factor Eyeless (Ey) as a candidate for regulating Notch signaling and found that without Ey, Notch signaling is reduced. In this work, we discover that cell signaling pathways that involve the receptor Notch and its ligand Delta function to regulate NB proliferation in *Drosophila* by regulating early temporal factor expression, and Ey may contribute to Notch's differential roles in NB lineages.

1749T **Glial-specific knockdown of a subunit of the ER membrane complex (EMC) impacts development and adult survival of *D. melanogaster*** Maria Jose Orozco Fuentes[1], Ines Riojas[2], Otoha Tatami[2] [1]Neuroscience and Biology, Lake Forest College, [2]Biology, Lake Forest College

The ER membrane protein complex (EMC) is believed to participate in crucial functions of the endoplasmic reticulum such as protein folding, insertion, and packaging. This complex consists of 8-10 subunits and it is evolutionarily conserved from yeast to humans. A previous study showed decreased expression of EMC4 in glia after a traumatic brain injury (TBI) in Drosophila. Consequently, we decided to study the effect of an RNAi knockdown of EMC4 in glia, which resulted in notable phenotypes, such as delayed development, strongly impaired locomotion, a substantially shorter lifespan of 5-6 days, and increase in protein aggregation. We conducted a temporally-restricted knockdown and observed that fruit flies with normal levels of EMC4 during development but an adult-specific knockdown do not exhibit the same severity of adult phenotypes. This suggests that EMC4 has an important role in glia during development that impacts organismal health during adulthood. Additionally, preliminary studies suggest changes in expression of markers of ER stress response. The dramatic effect of this development-specific glial knockdown together with the known role of EMC in protein biogenesis suggest that EMC4 plays a critical role in some developmental function of glia involving transmembrane proteins.

1750T **Disruption of the arylalkylamine Nacetyltransferase-like-7 gene*, AANATL-7*, identifies an additional metabolic pathway for histamine inactivation in *Drosophila melanogaster*** Lydia Cruce[1], Margaret Cubitt[1], Katie Westfall[2], Martin G Burg[2] [1]Cell & Molecular Biology, Grand Valley State Univ, [2]Biomedical Sciences, Grand Valley State Univ

In *Drosophila melanogaster*, 8 genes encoding arylalkylamine Nacetyltransferase-like enzymes have been identified that likely acetylate molecules involved in several biological processes, from aminergic neurotransmitter inactivation, melanin synthesis, to sclerotization of the cuticle. The arylalkylamine Nacetyltransferase-like-7 protein (encoded by the *AANATL-7* gene) has been shown to acetylate histamine *in vitro*, producing N-acetylhistamine (NAH) **(1)**. In *D. melanogaster*, histamine is a photoreceptor neurotransmitter and is also present in the CNS, likely functioning as a neuromodulator. Genes that contribute to histamine metabolism in photoreceptors include histidine decarboxylase (*Histidine decarboxylase*; histamine synthesis), β-alanyl synthase, (*ebony*; histamine conversion to carcinine), and β-alanyl hydrolase (*tan*; carcinine conversion to histamine) **(2).** We have detected *Hdc*-dependent histamine-like immunoreactivity in the secondary cells of male accessory glands where NAH has also been shown to be present **(3)**. Pre-absorption of a "histamine" antibody indicated it can be blocked

by histamine, carcinine, or NAH. In *ebony* mutants, the immunoreactivity is still present in secondary cells, suggesting that this immunoreactivity could be identifying NAH. Since AANATL-7 acetylates histamine *in vitro* **(1)** and is expressed in the accessory gland **(4)**, we generated *AANATL-7* mutants using a co-CRISPR/cas9 mutagenesis approach, using *ebony* as a visual marker. From 96 G_0 parents, 25 G_0 flies generated *ebony* progeny (ranging from 1-53% mutant progeny within each *ebony*-producing G_0 brood), from which single *ebony* flies were used to establish isogenic lines. Male flies from these isogenic lines were subjected to sequence analysis, which indicated that progeny from at least 7 isogenic lines contained frameshift mutations at the sgRNA-targeted site in the *AANATL-7* gene. Immunostaining *AANATL-7* mutant accessory glands with the "histamine" antibody indicated that the histamine-like immunosignal was mostly eliminated. This result suggests that the AANATL-7 protein acts as a histamine acetylase in male flies, as the *AANATL-7* mutations eliminate most of the immunoreactivity in the accessory gland and is therefore presumed to be detecting NAH. These results suggest that histamine acetylation is a novel step in histamine metabolism in *D. melanogaster*.

1. Dempsey et al (2015) doi: 10.1021/acs.biochem.5b00113 2. Borzyc et al (2012) doi:10.1242/jeb.060699 3. Chintapalli et al (2013) doi:10.1371/journal.pone.0078066 4. Gramates et al (2022) Genetics: 220 (4), iyac035

1751T **Exploring the Connection Between Circadian Rhythms and Sex-Specific Behavior in *Drosophila*** Brooke E Bascom[1], Anthony S DeLuca[1], Matthew A. Kocher[1], Daniela A. Key Planas[1], Marielise Torres[1], Michelle Arbeitman[2] [1]Florida State University, [2]Biomedical Sciences, Florida State Univ

Circadian rhythms govern daily activities in *Drosophila.* Here we investigate the relationship between sex-specific behavior and the circadian system by examining a set of neurons that express the transcriptional regulatory genes *clock (clk)* and *fruitless (fru)*. *Clk* encodes a transcription factor of the core molecular circadian pacemaker, governing the diurnal activity pattern. *fru P1* encodes male-specific transcription factors that are necessary and sufficient for male courtship behavior. *fru P1* expressing neurons are found in both males and females and underlie reproductive behaviors. Our previous single-cell RNA sequencing data revealed intersecting *fru P1* and *clk* (*fru* Ω *clk)* expressing neurons, present in both male and females. Here we examine individual *fru* Ω *clk* – expressing neurons in males and females over six circadian time points through confocal microscopy, using a multi-cassette FLP-out approach. In addition, we examine how the sex hierarchy regulates sex differences in *fru* Ω *clk* - expressing neuron number and morphology. We examine the role of *fru* Ω *clk* - expressing neurons in establishing circadian period length, sleep and activity, and reproductive behavior by activating and silencing these neurons. Using the Trans-Tango system that labels neuronal targets, we find *fru P1*+ targets of *clk*, Per+ targets of *fru P1*, and Per+ targets of the other sex hierarchy transcription factor encoded by *doublesex* (*dsx*). In addition, using a transcriptional reporter of neuronal activity called CRTC, we determine if mating changes male fru Ω clk neuron activity. Finally, we ask if conditions that result in a shortened period, through activation of fru Ω clk neurons, impact the molecular clock in a non-autonomous fashion.

1752T **The Role of F-box Protein FBXL20/CG9003 in Drosophila Synapse Development** Tianlu Wei, Lily Liu, Junghwan Kim University of Nevada,Reno

The ubiquitin proteasome system (UPS) is crucial for protein homeostasis in the nervous system. The F-box proteins are one of the fundamental components in the UPS by playing a role in substrate selectivity. Dysregulation of the F-box proteins can lead to detrimental effects on synaptic connectivity and plasticity in neurodegenerative conditions. In this work we characterized the synaptic function of *CG9003*, the *Drosophila* ortholog of human F-box proteins--FBXL20 and FBXL2, in the larva neuromuscular junction (NMJ) and the larval sensory circuit. We found that *CG9003* mutants exhibited a reduction in both bouton number and total length in NMJ. Furthermore, *CG9003* mutations caused decrease in synaptic connectivity of the larval sensory circuit, which was revealed by a reduction in the synaptic GFP reconstitution across synaptic partners between the sensory neuron and its postsynaptic projection neurons. These findings suggest a role of *CG9003* in synapse development. Interestingly, FBXL20 and FBXL2 physically interacts with YPEL proteins in human cells. *YPEL3* belongs to the Yippee-like gene family, which is conserved across eukaryotic species from yeast to humans. We found that CG9003 physically interacts with dYPEL3 in *Drosophila*. Previous research from our laboratory has demonstrated a similar reduction in the sensory synaptic connectivity by *dYPEL3* mutations. We further found that *dYPEL3* mutations caused similar NMJ defects as in *CG9003* mutants. In summary, our findings suggest that *CG9003* and *YPEL3* operate within the same pathway to regulate synapse development.

1753T **Life, Death, & Cannibalism** Emily Siff, John Carlson Molecular, Cellular, & Developmental Biology (MCDB), Yale

Where there is life, there is death. Death can occur due to a variety of factors, such as lack of resources, disease, predation, and old age. Although death may be a common occurrence, how animals sense and respond to the dead of their own kind (conspecifics) remains curiously unexplored. Utilizing behavioral, genetic, neural, and computational approaches in the fruit fly (*Drosophila*), this work seeks to elucidate fundamental questions about how animals sense and respond to the dead. While death is often viewed as an aversive state, this research uniquely studies contexts in which death has a positive valence, thus generating entirely new findings, as well as re-contextualizing previous work Zooming out, this research contributes to a larger,

interspecies body of work that has begun to suggest that life, death, and cannibalism are closely intertwined.

1754T **Connecting temporal patterning in neural progenitors to neuronal identity in Drosophila Visual System** Asif Ahmad A Bakshi[1], Claude Desplan[1,2] [1]Center for Genomics and Systems Biology, New York University Abu Dhabi, [2]Department of Biology, New York University

Neural progenitors in the Drosophila visual system are temporally patterned to produce a variety of neuronal types. Apart from temporal patterning, the neuronal diversity is driven mainly by spatial patterning, and binary cell fate decisions mediated by Notch signaling. The integration of all three mechanisms specifies around 200 neuronal types in developing Drosophila visual system. Temporal patterning is primarily driven by the sequential and overlapping expression of a series of transcription factors called temporal TFs (tTFs). Most of these tTFs express in NBs and pass on to newly born neurons, however they are not maintained throughout development. tTFs activate the expression of a set of transcription factors called Selectors, whose sustained expression is maintained in the neurons and they specify the molecular and functional identity of neurons downstream of temporal factors. However, how do tTFs pass on the early specification program in NBs to regulate and maintain neuronal identity via cell type-specific selectors genes is not clear. I am using DamID based genomics followed by its integration with the single cell multiome data to answer this question. Identifying potential target genes of tTFs, particularly Selectors, in cell-type specific manner will be the first step towards getting a deeper understanding of the underlying mechanisms driving neuronal diversity in Drosophila visual system.

1755T **Non-ionic GluR signaling remodels DLG to induce rapid retrograde homeostatic plasticity** Chengjie Qiu[1,2], Yifu Han[3], Christine Chen[3], Pragya Goel[3], Sarah Perry[3], Dion Dickman[4] [1]Neuroscience Graduate Program, University of Southern California, [2]Department of Neurobiology, University of Southern California, [3]University of Southern California, [4]Department of Neurobiology, Unviersity of Southern California

Synapses must be resilient to the challenges they confront during development, growth, disease, and aging. At the glutamatergic *Drosophila* neuromuscular junction (NMJ), acute pharmacological perturbations of glutamate receptors (GluRs) by philanthotoxin-433 (PhTx) can be counteracted by enhanced presynaptic neurotransmitter release to maintain synaptic strength, a process called presynaptic homeostatic potentiation (PHP). Much is now known about the genes and mechanisms in the presynaptic neuron that enable the expression of PHP. However, how retrograde PHP signaling is rapidly induced in the postsynaptic compartment remains enigmatic. Importantly, recent evidence suggests that perturbation of ionic flow through GluRs or reductions in postsynaptic Ca^{2+} influx are insufficient to induce rapid PHP expression. Here, we combine molecular genetics, electrophysiology, and confocal and super-resolution microscopy to suggest that conformational signaling through GluRs is necessary for the induction of rapid, retrograde PHP. First, we find that pharmacological antagonism of GluRIIA-containing receptors provokes changes in GluR organization, which is propagated throughout the postsynaptic apparatus. Indeed, preliminary data suggests these structural changes to the postsynaptic compartment are necessary for the presynaptic adaptations that characterize PHP, including active zone remodeling and enhanced neurotransmitter release. Second, we identify Discs large (Dlg), from a CRISPR/Cas9-based genetic screen, as a key postsynaptic scaffold that is targeted for conformational signaling and that is selectively required for rapid PHP expression. Finally, we interrogated whether synaptic activity itself was necessary for PHP inductive signaling. Surprisingly, we find that homeostatic remodeling of both the pre- and post-synaptic compartments persists following pharmacological antagonism of GluRs in the absence of neurotransmission. Altogether, our findings support a model in which acute perturbation of GluRs initiates conformational signaling, which is propagated throughout the postsynaptic apparatus, transmitting retrograde information that is necessary to induce homeostatic plasticity to presynaptic release sites.

1756T **Neural origin of a female-specific sexual behavior in *Drosophila santomea*** Minhao Li[1], Dawn Chen[1], Ian Junker[1], Aaron Comeault[2,3], Daniel Matute[2], Yun Ding[1] [1]Biology, University of Pennsylvania, [2]University of North Carolina at Chapel Hill, [3]Bangor University

Courtship interactions are remarkably diverse in form and complexity among species and intrinsically dynamic. How neural circuits evolve to encode new adaptive social behaviors that function in the appropriate sensory and motivational contexts is not known. Here we leverage *Drosophila* species as an emerging neural comparative model and a recently evolved female sexual behavior to explore the circuit basis underlying the origin of new social behaviors. Females in *D. santomea* exhibit wing spreading as a functional receptive response to male courtship songs – an ancestral behavior that has been lost in the lineage of *melanogaster* subgroup but re-emerged after the divergence from the sibling species *D. yakuba* within the recent 0.4 million year. We hypothesized that wing spreading originated through modification of pre-existing female sexual circuits that express the sex determination gene *doublesex*. Therefore, we developed genetic tools that specifically label and manipulate *doublesex* neurons for functional comparison in *D. santomea*, *D. yakuba*, and the model species *D. melanogaster*. Optogenetic activation of *doublesex* neurons across species and the co-occurence of wing spreading with the conserved female

behavior vaginal plate opening together suggests that vpoDN, a pair of descending neurons that integrates male courtship song stimuli and female internal state to drive vaginal plate opening, drives robust wing spreading in *D. santomea* but not in *D. yakuba* or *D. melanogaster*. Interestingly, *D. santomea* vpoDN neurons exhibit a species-specific arbor that innervates wing neuropil. Surprisingly, vpoDN activation elicited wing spreading in a very small number of females in *D. melanogaster*, and its efficacy to elicit wing spreading drastically increased at a higher developmental temperature, supporting the existence of latent circuits that plastically potentiate wing spreading. Overall, we identified the neural mechanism of a newly originated female behavior in *D. santomea* and revealed the existence of a latent and plastic ancestral circuit in *D. melanogaster*. Our study shows that new social behaviors may arise from the assembly of key circuit nodes that interpret social contexts with latent motor programs, highlighting how ancestral circuits potentiate and shape behavioral evolution.

1757T **Clearance of cell corpses in the phagocytosis-deficient *Drosophila* brain** Cheng Yang (Jason) Shi[1], Guangmei Liu[2], Iqra Amin[2], Kim McCall[2] [1]Biology, Boston University, [2]Boston University

Billions of cells die per day to maintain homeostasis during development, growth and aging. Physiologically, dead cells are rapidly engulfed and degraded by phagocytes through phagocytosis. In the central nervous system, glial cells play a pivotal role in eliminating dead cells. Deficits in phagocytosis can result in different pathologies, including autoimmune and neurodegenerative diseases. In *Drosophila*, Draper (Drpr) is a crucial phagocytic receptor on the surface of glia to recognize dead cells. Our lab previously found that knocking down *drpr* in glia lead to persistence of neuronal corpses, actively dying glia, and age-dependent neurodegeneration. Notably, these neuronal corpses are neurons that died during larval and early pupal development, and the number of neuronal corpses remains constant as fly aged. However, whether neurons that die after developmental stages are engulfed and cleared is not known. Unlike persisting neuronal corpses in the *drpr*-deficient flies, glia are actively dying and the number of glial corpses decreases over time suggesting they are cleared independently of *drpr*. However, the mechanism by which these glial corpses are cleared is still unknown. Using markers to detect engulfment, we are investigating whether neurons and glia that die during adulthood are cleared by fellow healthy glia in the absence of *drpr*.

1758T **A microRNA screening reveals miR-33 as a modifier of TDP-43 toxicity in flies** Swapnil Pandey, Alfonso Martin-Pena, Deepak Chhangani, Shivam Kaushik, Diego Rincon-Limas Neurology, University of Florida

Abnormal distribution and phosphorylation of TAR DNA-binding protein 43 (TDP-43) are hallmarks of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Although the mechanisms underlying TDP-43 proteinopathies are largely unknown, recent evidence suggest that aberrant microRNA (miRNA) biogenesis or function might be linked to TDP-43 neurotoxicity. Indeed, FTLD/ALS patients display abnormal expression of at least 23 miRNAs. Since the mechanisms controling these miRNAs and the identification of their bona fide targets remain to be elucidated, the overall impact of miRNAs on TDP-43 proteinopathies is largely unknown.

To shed light on this issue, we recently screened a library of 107 *Drosophila* miRNAs in a fly model expressing human TDP-43^{M337V}. This library contains miRNAs that are highly conserved throughout evolution and was recently created with an attP-based UAS vector to ensure identical miRNA expression levels from all constructs. We found that most miRNAs do not modify the TDP-43^{M337V}-induced eye phenotype or lead to severe developmental eye phenotypes when misexpressed on their own. However, we found that over-expression of mir-33 dramatically suppressed mutant TDP-43 toxicity in the *Drosophila* eye. This is relevant because human mir-33 is downregulated in FTDL patients with TDP-43 pathology. Importantly, mir-33 does not rescue eye phenotypes in fly models of other proteinopathies, suggesting that the robust ability of mir-33 to block TDP-43 toxicity is highly specific. Interestingly, we have tested several mir-33 targets for their potential to mimick the rescue of TDP-43 phenotype when knocked down by expression of their especific RNAi contruct. These results led us investigate the role of extracellular matrix (ECM) glycoproteins involved in cell adhesion, signaling, and neurite outgrowth. Thus, we hypothesize that TDP-43 pathology triggers mir-33 dysfunction, which results in ECM alterations. To test this, we are currently assessing the role of mir-33 in the fly CNS to obtain a better understanding of the TDP-43 pathology and its association with ECM abnormalities. This work is supported by the NIH grant R01AG059871 to DERL.

1759T **Identifying the genetic basis of olfactory learning and memory skills in *Drosophila melanogaster*** Reiley Heffern, Jamie Baumann, Victoria Hamlin, Elizabeth King Biological Sciences, University of Missouri - Columbia

Learning and memory skills are necessary for organisms to adapt to their changing environment. For example, if a population migrates, those organisms may use olfactory stimuli to learn and remember where food or threats are in their new environment. Studies have shown that these skills are influenced in part by one's genetic makeup. Learning and memory abilities are complex traits, meaning that multiple genes and the environment contribute to these skills. In our investigation using nine recombinant inbred lines (RILs) of *Drosophila melanogaster*, we are aiming to identify which genetic variants are associated with higher performance on learning and memory tasks.

Operant conditioning is the process by which organisms learn to predict an outcome from a certain stimulus. We utilize operant conditioning to train flies of each RIL to associate a certain odor with a sugar reward. We then use Y-mazes to present them with a choice between the odor we conditioned them to (the positive stimulus) and an odor that is not associated with a reward (the negative stimulus). We conduct this testing on one group of flies immediately after conditioning to test their learning skills, and again four hours after conditioning on the other group to test their short-term memory skills. At the conclusion of each test, we collect and count the number of flies that chose correctly, chose incorrectly, and did not choose. We then quantify these results for each RIL by calculating a performance score. After completing three replicates of testing, we will computationally analyze our phenotyping data to identify quantitative trait loci (QTLs) that are linked to learning and memory skills. Then, we plan to look at those QTLs and analyze the significance of any differences in haplotype frequency at these QTLs in each RIL. If RILs that have high performance scores have a high frequency of a certain haplotype at a QTL, and RILs that have low performance scores have a low frequency of that haplotype at the same QTL, that haplotype at that QTL may contribute to higher learning and memory abilities.

1760T **Identification and Characterization of Sleep-Regulating Neuronal Subsets within the Central Complex of *Drosophila melanogaster*** Preeti Sundaramurthi[1], Tanya Wolff[2], Nan Chen[2], Divya Sitaraman[1], Gerald M Rubin[2] [1]Psychology, California State University, East Bay, [2]Janelia Research Campus, Howard Hughes Medical Institution, Ashburn VA

Sleep is a fundamental, highly conserved behavioral state that is important for the survival of most animal species ranging from those with few neurons, like jellyfish, to those with brains and complex nervous systems, like humans. The ability of an animal to switch between sleep and wakefulness states, and to persist in the wakefulness state to carry out activities such as mating and feeding is a hallmark of the sleep-wake behavior. Discrete populations of neurons have been identified in multiple organisms such as fish, worms, and flies that either increase (sleep-promoting) or decrease (wake-promoting) the animals' propensity to sleep. Interactions between these neurons is critical for the sleep-wake behavior, but the precise mechanisms of communication between these identified sleep-regulating neurons is poorly understood.

The fruit fly, *Drosophila melanogaster*, has proven to be an excellent model to study the circuit basis of sleep. A specific brain region called the Central Complex has been implicated in sleep regulation. Using a library of split-GAL4 lines targeting specific neuronal cell types within the central complex we have identified several neurons that are critical to sleep onset and maintenance. We will present the results of the screen and discuss the role of a specific class of sleep promoting neurons known as PFGs that innervate multiple regions within the central complex. PFGs regulate sleep in a sexually dimorphic way using a combination of neurotransmitters and neuropeptides. We will explore the function of these neurochemicals and present hypotheses related to how PFGs integrate within existing sleep regulatory circuits within the central complex.

1761T **Are the functions of Netrin and Frazzled conserved among insects?** Piyasi Ghosh, Ben Wadsworth, Tim Evans BISC, University of Arkansas

Axon guidance in bilaterians is often controlled by signaling pathways in the nervous system that determine the fate of the axons, for example, if they would cross the midline or not. One such pathway involved is a ligand-receptor pair Netrin (Net) and Frazzled (Fra), which is also known as the "attractive pathway". Net and Fra together promote midline crossing of axons in insects and other bilaterians. Orthologs of the pathway receptor Fra in insects, is Deleted in Colorectal cancer (DCC) in vertebrates, and UNC-40 in *Caenorhabditis elegans.* How well are the regulatory roles of these proteins conserved is not well understood. Hence, this project aims to compare the direct evidences of midline attractive roles of the Frazzled receptor in the flour beetle (*Tribolium castaneum*) and fruit fly (*Drosophila melanogaster*) using CRISPR/Cas9 gene editing tools. In this project we rescued the role of Frazzled from Drosophila (DmFra) in promoting midline crossing of axons in Drosophila using an HA-tagged Frazzled from Tribolium (TcFra).

1762T **Insights into the aging brain through unraveling the development and transcriptional signature of polyploid cells** Deena Damschroder, Jenny Sun, Laura Buttitta University of Michigan

Polyploid cells contain multiple copies of chromosomes and are derived various ways, including a non-canonical cell cycle known as an endocycle, which involves DNA synthesis without cytokinesis. Numerous tissues are known to contain polyploid cells, including the brain. In fact, in mammals there is a well-established correlation between polyploid neurons in the aging brain and neurodegeneration. Despite this correlation, the genes responsible for the formation of polyploid cells in the aging brain and the functional role of these cells is unknown.

In our previous work, we observed the accumulation of polyploid cells in the adult brains of multiple genotypes and species of aging Drosophila. Here, we expand on that work examining how known lifespan modifiers influence the accumulation of polyploid cells in the aging brain. We find that the accumulation of polyploidy with age can be impacted by diet and temperature-dependent lifespan extensions, but that it does not proportionately scale with lifespan extensions. This suggests

that the genetic networks involved in neuronal and glial polyploidy in the adult brain are complex, and may be impacted by both developmental and adult physiological processes.

To understand the consequences of polyploidy in the brain, we are examining the transcriptional signature of polyploid neuronal and glial cells in the adult brain. From this work we aim to identify molecular markers of polyploidy in these broad cell types and identify genetic regulatory networks that may be activated or repressed in these cells to impact their cellular functions. Collectively, we aim to shed light on the causes and consequences of polyploidy in the brain under normal physiological conditions

1763T **Effects of peripheral vs local immune activation on larval neuroblast proliferation** Omina Nazarzoda[1], Michelle Bland[2], Sarah Siegrist[3] [1]University of Virginia, [2]Pharmacology, University of Virginia, [3]Biology, University of Virginia

Healthy brain development depends on optimal environmental conditions such as food, temperature, and protection of fetus and mother from pathogens. In resource-poor environments, where children and mothers are constantly exposed to pathogens and lack accessible health care, chronic immune activation is associated with disrupted growth and cognitive development in children. One way to predict neurodevelopmental disruption is to look at the earliest stages of neural stem cell proliferation that makes the brain. We investigate if neural development is affected by genetic immune activation in different tissues/cells. We use *Drosophila* neuroblast proliferation as a proxy for neural development, and expression of a mutated Toll^{10b} receptor for a constitutive activation of immune signaling in fat body or glial cells. We observe reduced neuroblast proliferation in larvae expressing Toll^{10b} in larval fat body (r4-Gal4 > UAS-Toll^{10b}). We also measured the mRNA levels of antimicrobial peptide Drosomycin (Drs), which is induced in response to Toll pathway activation. We find a significant increase in Drs mRNA levels in whole larval brain when Toll^{10b} is expressed in glia (repo-Gal4), but inconsistent in fat body (r4-Gal4 and cg-Gal4 driver). Taken together, these results suggest that brain development is impacted by the activation of immune signaling in fat body likely via secreted factors or via activation of hemocytes. Activation of immune signaling in glial cells, on the other hand, shows that glia are competent to respond to Toll^{10b} expression by inducing Drs, suggesting local effects of immune signaling on larval brain development.

1764T **Investigating the Role of the ER Integral Membrane Protein Jagunal in Neural Development in *Drosophila*** Nina Nicole Marcelo[1], Maria L Mendoza Aragon[1], Judy Abuel[2], Blake Riggs[2] [1]Biology, San Francisco State University, [2]San Francisco State University

Cell division is an important occurrence in life and is essential for the creation of all multicellular organisms. Although we understand how cells divide during mitosis, it is still unknown how cells adopt their cell fate and become specialized cells. Recent studies have shown that the highly conserved endoplasmic reticulum (ER) transmembrane protein, Jagunal (Jagn), is necessary for the asymmetric cell division (ACD) of the ER during mitosis in early *Drosophila* embryos. Preliminary data shows that Jagn-deficient larval brains display a shift towards stemness with an over proliferation of neuroblasts. This leads us to examine the role that Jagn plays in neural development. We hypothesize that Jagn is involved in the regulation of cell differentiation allowing cell fate determinants to orient properly which is vital in the development of *Drosophila* brain. Jagn mutants display lethality in 1st instar, thereby making it difficult to properly examine the role of Jagn in larval brain development. In order to test our hypothesis, we dissected 3rd instar larval brains of a Jagn-RNAi line driven with a Delta-Gal4 driver to investigate whether Jagn is necessary in the development of the *Drosophila* larval brain. Our results reveal that Jagn-RNAi 3rd instar larva display a significantly smaller brain size compared to the controls. Specifically, inhibition of Jagn, showed a reduction of the ventral nerve cord (VNC) in the larval brain. Based on this result, we sought to examine any defects in larval feeding behavior by performing a feeding assay. However, our results show that Jagn-RNAi larvae consumed around the same amount of food as our control. In order to understand the *Jagn*-induced lethality, we immunostained larval brains with an apoptotic marker, DCP1, to examine if Jagn-RNAi brain cells experience higher levels of cell death. We found that indeed, Jagn-RNAi larval brains displayed higher levels of DCP1, suggesting that Jagn is necessary in developing the brain and in maintaining homeostasis and cell populations in tissues. Future direction will focus on staining cell fate determinants that play a role in neurodevelopment during larval brain development, as well as perform behavioral assays to assess the locomotor, learning, and memory deficits. Furthermore, our findings will contribute to the understanding of neurogenesis and cell fate differentiation during early development of *Drosophila* brains.

1765T **Investigating cell type-specific requirements for the RNA exosome within the brain through study of Pontocerebellar Hypoplasia Type 1b disease mutations in *Drosophila*** Lauryn A Higginson[1], Derrick J Morton[2] [1]University of Southern California, [2]Biological Sciences, University of Southern California

Accurate post-transcriptional regulation of gene expression is critical for proper neuronal development and function. Many of these highly coordinated post-transcriptional regulatory events are mediated by an evolutionarily conserved and ubiquitously

expressed RNA processing complex, the RNA exosome. Despite the broad, essential roles of the RNA exosome, missense mutations in genes that encode subunits of this complex cause neurological disorders characterized by distinct cell type defects. Recessive mutations in RNA exosome subunit gene *EXOSC3* cause a devastating human neurodevelopmental disorder, Pontocerebellar Hypoplasia Type 1B (PCH1b). PCH1b is characterized by defective development and atrophy of the brainstem and cerebellar structures. Why mutations in genes encoding structural subunits of the RNA exosome preferentially affect cells within specific brain regions and result in distinct pathology is unclear, but this suggests that specific tissues depend on the RNA exosome more than others. To understand whether regulatory programs in specific cell types within the brain require unique functions of the RNA exosome complex, we have generated an allelic series of pathogenic variants in the RNA exosome cap subunit, *EXOSC3* (fly *Rrp40*) in *Drosophila* modeling severe (Rrp40-G11A) or moderate (Rrp40-G146C) disease. My preliminary studies show that PCH1b disease-causing Rrp40 variants cause a spectrum of organismal phenotypes, including reduced viability, behavioral defects, and defects in brain morphology. In addition, single-nuclei RNA-sequencing analysis of each *Rrp40* mutant fly reveals distinct gene expression profiles for cell subpopulations. Furthermore, differential expression analysis on these distinct cell subpopulations shows aberrant accumulation of functionally important neuronal transcripts, including *Arc1*, a key regulator of synaptic plasticity. To validate our single-nuclei datasets, we employed quantitative mRNA imaging technology throughout the entire *Drosophila* brain via Hybridization Chain Reaction (HCR) RNA-FISH to visualize defects in mRNA expression and localization, including *Arc1*. In sum, this work represents the first cell-specific analysis of pathogenic RNA exosome mutations in an intact nervous system *in vivo* and, thus, provides insight into whether the RNA exosome is vital for the proper function of all cell types within the brain or whether it has specialized roles in neuronal cell subtypes.

1766T **Investigating the Cross-regulation of DATILÓGRAFO and NOCFLY in Decision Making** Amina Jahan Shammo[1], Ornella Meko[2], Kexin Zhang[3], Aravindan Krishnan[1], Rui Sousa-Neves[2], Claudia Mizutani[1] [1]Biology, Case Western Reserve University, [2]Genetics and Genome Sciences, Case Western Reserve University, [3]Case Western Reserve University

The transcription factor DATILÓGRAFO (DATI) is required in a neural circuit in the brain that decides whether a female accepts or rejects male courtship. Recently, we identified computationally the targets of DATI that are conserved from *Drosophila* to humans. Interestingly, 20% of these genes are candidates for Alzheimer's Disease (AD) and/or interact with the central gene of familial AD, the Amyloid Precursor Protein (APP). AD is a disease mostly known for its devastating effects on memory, and it also impairs decision making. Among the DATI targets identified that interact physically and/or appear as candidates for AD, we find *nocfly* (*CG44422*). Here we provide evidence that, like DATI, NOCFLY is required for female acceptance. NOCFLY (CG44422), is the homolog of the Potassium voltage-gated channel interacting proteins 1/4 (KCNIP1/4) that acts as Calcium-dependent transcriptional repressor and a regulator of Calcium and Potassium channels. These data argue that DATI is required for the expression of *nocfly*, which is turn represses a set of DATI targets. To further investigate this possibility, we searched computationally for candidate genes to be regulated by NOCFLY. Surprisingly, 89% of the DATI targets are NOCFLY candidate targets, suggesting an extensive cross-regulation between DATI and NOCFLY. This result suggests that NOCFLY may dampen the expression of a large group of DATI targets. To test this possibility, here we analyze the effects that the loss of DATI or NOCFLY have on the transcriptome of the cholinergic neurons that make these decisions. We generated flies expressing RFP in the cells of interest in a normal background, and in cells with loss of expression of either DATI or NOCFLY and used FACS to sort these cells for RNAseq analyses. We expect this approach will enable us to gain insights into the gene network and co-regulation of target genes by these two transcriptions factors.

1767T ***Pax3* expression within neural crest is essential for differentiation of sacral progenitors that form pelvic autonomic neurons** Michelle Southard-Smith[1], Edom Seyoum[1], Ysabel Gomez[1], Karen Deal[1], Zunyi Wang[2], Dale Bjorling[2] [1]Genetic Medicine, Vanderbilt University Medical Center, [2]Surgical Sciences, University of Wisconsin Madison

Innervation of the lower urinary tract (LUT) is required for normal micturition patterns. Motor neurons that mediate contraction of the urinary bladder are part of the autonomic nervous system that originates from the neural crest. In rodents, these neurons are aggregated into major pelvic ganglia (PG) just dorsolateral to the bladder neck. PG are formed from sacral level neural crest whose migration paths into the fetal urogenital sinus have been recently described. However, knowledge of the genes that regulate PG neurogenesis has been lacking. To identify candidate factors during development of PG, we assessed transcription factor expression patterns in lower urinary tracts of fetal mice. This analysis identified a prominent pulse of *Pax3* expression during PG neurogenesis and subsequent hybridization chain reaction in situ identified *Pax3* mRNA in differentiating PG neurons. Global loss of *Pax3* causes perinatal lethality that prevents postnatal analysis of LUT function. We devised crosses using a conditional allele *Pax3*$^{tm1.1Sjc}$ (*Pax3*^{flox}*, after cre Pax3*^{cKO}) to delete *Pax3* from neural crest progenitors that give rise to pelvic autonomic neurons. Using a cre line that is prominently expressed in neural crest progenitors (Tg(Sox10-cre)1Sout) we examined whether neural crest expression of *Pax3* is required for normal development of PG, bladder innervation, and bladder function. Offspring from *Pax3*^{flox} x Tg(Sox10-cre) crosses (*Pax*$^{NC\text{-}cKO}$) lack *Pax3* in multiple neural crest lineages including melanocytes, peripheral nervous system, and autonomic neurons. In fetal stages, *Pax*$^{NC\text{-}cKO}$ mice

have normal-sized aggregates of sacral NC progenitors that colonize the fetal urogenital sinus. However, differentiation of PG neurons is delayed in fetal *Pax3* $^{NC\text{-}cKO}$ mice and postnatally the PG of these mutants are smaller, with abnormal morphology. *Pax3* $^{NC\text{-}cKO}$ mutants survive to adulthood, exhibit altered voiding patterns, and functional studies of bladder contractility using electrical field stimulation on bladder muscle strips found reduced bladder contractility in these mutants. These studies demonstrate that *Pax3* plays an essential role in differentiation of sacral neural crest that form the PG and establishes a novel, viable mouse model for further study to define essential gene regulatory networks in PG neurogenesis.

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1768T **Epistatic mutations in *Ssc4d* and *Kpna3* regulate age dependent hyperactivity in mice.** Yehya Barakat[1,2], Sean Deats[3], Tom Sproule[3], Marina Santos[3], Jacob Beierle[3], Vivek Kumar[3] [1]Kumar Lab, The Jackson Laboratory, [2]Tufts University, [3]The Jackson Laboratory

The NCHS estimates that 8.56% of children in the United States are diagnosed with developmental disorders. It is therefore critical to understand the neurobiological mediators of developmental disorders to improve patient outcomes. Through ENU mutagenesis our lab has generated a mutant mouse line (Crichton ENU) that exhibits age dependent hyperactivity starting at 6 and plateauing at 20 weeks of age. Quantitative trait loci analysis (QTL) identified 2 genome wide significant QTLs and identified missense mutations within *Ssc4d* and *Kpna3* as causal. Both mutations are needed to express the hyperkinetic phenotype, demonstrating a novel epistatic relationship between the understudied scavenger receptor *Ssc4d* and nuclear import factor *Kpna3*. This was confirmed by generating a *Ssc4d*$^{mut/mut}$ and *Kpna3*$^{mut/mut}$ CRISPR mouse line (Crichton CRISPR) which had a similar pattern of hyperactivity to the original ENU line. To interrogate the mechanism of this epistasis, we examined protein stability, protein binding, and RNA expression in the brain. We observe no destabilization in the mutant proteins but analysis of differential KPNA3 binding partners revealed a general loss of canonical binding partners important for its nuclear import capabilities.

We also investigated transcriptomic differences between young (4-week-old) and adult (20-week-old) Crichton and wildtype mice in the prefrontal cortex (PFC), hippocampus, and choroid plexus using bulk RNAseq. *Ssc4d* is highly expressed in the ChP, where differentially expressed gene (DEG) analysis and gene ontology enrichment analysis revealed decreases in cell organization genes in old mutants. DEG analysis revealed cilia and developmental synaptic pathways being impacted in Crichton CRISPR. Finally, PFC DEG analysis highlighted myelination and oligodendrocyte differentiation downregulation in the young mutants. These results reveal a slew of developmental changes that will enable us to interrogate this epistatic relationship on a mechanistic level. Additional future directions will involve examining choroid plexus structurally and functionally and using viral tools to examine the role of SSC4D in the brain. By pursuing these gene expression changes it will lead us to closer to understanding how *Kpna3* and *Ssc4d* interact to promote motor control development.

1769T **Role Of CRISPR-Cas9 Screens in Elucidating the Gene Functionality in Patients Derived Induced Pluripotent Stem Cells (iPSCs) For Neurodegenerative Disorders** Ammara Talib Biomedical Engineering, Near East university north Cyprus

Neurodegenerative illnesses are wreaking havoc on sufferers and the global economy. The number of patients is continually increasing as life expectancy rises. These disorders are caused by nerve cell degeneration or loss of function. There are currently certain medicines that may alleviate some physical and mental distress, but we cannot slow or eliminate the disease. One major impediment to developing medicines is our lack of understanding of the underlying mechanisms. iPSCs have emerged as key participants in disease modeling and drug development, providing a sustainable source of human neurons for research. In this abstract, we discuss the critical function of CRISPR Cas screens within the iPSCs paradigm for elucidating the processes underlying neurodegenerative disorders that are required to comprehend the molecular mechanism at the functional level and to therapeutically target relevant genes. A revolutionary development has been the implementation of CRISPR-Cas9 technology for functional genomic screening. This is a flexible tool to investigate gene interactions, functions, and their effects on diverse biological processes, illnesses, and prospective therapeutic approaches. With the amazing success of the CRISPR Cas gene editing system in driving clinical research, there is a great deal of promise for the use of CRISPR screens in neurodegenerative illnesses. CRISPR screens are especially effective when combined with induced pluripotent stem cell technology, which allows for the generation of multiple cell types, including neurons and glia, as well as brain organoids, from cells taken from patients. CRISPR RNA interface, oligonucleotides and cDNA overexpression libraries were previously utilized to research loss and gain of function. CRISPR screens can aid in the identification of possible therapeutic targets. By carrying out loss of function or gain of function experiments Researchers can identify the genes that cause the disease by screening iPSCs-derived neurons. CRISPR-based genetic screening in human neurons produced from iPSCs could identify crucial variables driving neurodegeneration and the selective vulnerability of certain neurons.

1770T **Investigating the Role of Huwe1 and Fbxo16 in Neurogenesis** Haeli J Lomheim, Banu Saritas, Elena Silva Biology, Georgetown University

Our current knowledge of the regulation of neurogenesis primarily centers around the transcription factor cascade, with limited understanding of the role of protein degradation. Understanding neurogenesis at all levels is essential for the development of treatments for neurodegenerative diseases, neurodevelopmental disorders, and traumatic central nervous system (CNS) injuries. In an effort to better understand the role of protein degradation in neurogenesis, we are analyzing the function of two E3 ligases in Xenopus laevis: the F-Box protein Fbxo16 and the HECT, UBA, and WWE domain protein Huwe1. E3 ligases are ubiquitin proteins that are best known for targeting other proteins for degradation through poly ubiquitination and the 26S proteasome pathway. Fbxo16 is expressed in the neural plate and with gain and loss of function studies, we found that it is required for neuron formation and the formation of anterior neural structures. We sought to identify Fbxo16 target proteins via Mass Spectrometry of co-immunoprecipitated proteins and identified Huwe1. With morphant and half-life analysis, we are examining the role of Huwe1 and the mechanism of action of Fbxo16. Together our research indicates that surprisingly, these two E3 ligases play a role in stabilizing Neurogenin 2 thereby increasing neurogenesis.

1771T **Retinal neuron regeneration in zebrafish (Danio rerio) when TGFβ signaling is manipulated** Julinette Gines-Garcia[1], Daniela Sedano[2], Randi Marshall[2] [1]Biological Sciences, Goucher College, [2]Goucher College

Zebrafish have a greater ability to regenerate neurons in the central nervous system than many vertebrates, including mammals. We are using zebrafish (*Danio rerio*) to study the cell cycle dynamics of Müller glial cells (MGs) in retinal neuron regeneration. In zebrafish, MGs are responsible for initiating the regenerative process once they detect damage. MGs activate, dedifferentiate, generate neural progenitors, and then re-differentiate into MGs. Previous studies showed that fish with mutations in TGFβ signaling co-repressors (Tgif1 and Six3b) exhibit less regeneration but express higher ascl1a, a target of TGF β signaling and a pro-neural transcription factor required for some retinal neuron regeneration. Thus, we are particularly interested in the balance between TGFβ corepressors and ascl1a expression during neuron regeneration. We induced acute light lesions that target photoreceptors neurons and examined regenerative cellular processes in fish with increased TGFβ (*tgif1*^{fh258}*;six3b*^{vu87}), and a rescue condition with reduced functional Ascl1a (*tgif1*^{fh258}*;ascl1a*$^{t25215/+}$). We quantified MG activation, progenitor proliferation, and neuron regeneration to determine which phase of regeneration was disrupted in previous studies. Preliminary results suggest that at 1 day post lesion (dpl) MG activation is reduced in *tgif1*^{fh258}*;six3b*^{vu87} fish, and at 3dpl *tgif1*$^{fh258/fh258}$ fish have fewer proliferating cells than wild type and *tgif1*^{fh258}*;six3b*^{vu87} fish. At 3dpl, fish with the ascl1^{t25215} allele exhibited increased proliferation suggesting this can rescue the proliferation defect in *tgif1*$^{fh258/fh258}$ fish. By understanding the basic biology of retinal neuron regeneration in zebrafish we can uncover pathways and genes that can be targeted in biomedical research.

1772T **Functional analysis of two alternate Lef1 isoforms that may have distinct roles in regulating hypothalamic neurogenesis and innate stress-response behavior** Guangning Wang[1], Priscilla Figueroa[2], Richard Dorsky[1] [1]Neurobiology, University of Utah, [2]University of Utah

The Wnt/β-catenin signaling pathway transcription factor Lymphoid enhancer factor 1 (Lef1) has been demonstrated to regulate hypothalamic neurogenesis and stress-related behaviors in mice and zebrafish. Specifically, lef1 null mutant zebrafish fail to generate Crhbp+ neurons in the caudal hypothalamus and show decreased exploratory behavior. Different Lef1 protein isoforms have been described in animal brain regions, but their roles have not yet been explored. Alternative splicing creates Lef1 isoforms with a highly-conserved C-terminal N- or B-tail, and we are investigating whether these tail sequences function differentially in regulating neurogenesis and behavior.

Overexpression of human LEF1 N-tail isoform-encoding mRNA in zebrafish embryos induces abnormal dorsal axis formation, while B-tail encoding mRNA induces brain patterning defects, suggesting a potential difference in Wnt-dependent activity. Previous research in mice showed higher expression of B-tail-encoding mRNA in the embryonic brain, with a switch to higher expression of N-tail-encoding mRNA in adults, indicating stage-dependent regulation during development. In addition, a nonsynonymous human SNP in the final LEF1 exon affects a potential phosphorylation site (Thr369Met) unique to the B-tail. The frequency of this SNP varies significantly in different ethnic groups, with the highest levels found in Latin American and East Asian populations, and it has previously been linked to autoimmune disease in Koreans.

To determine if the B- or N-tail Lef1 isoforms are uniquely required for hypothalamic neurogenesis and behavior, we used CRISPR/Cas9 mutagenesis to generate isoform-specific zebrafish mutant lines. Our preliminary analysis indicates that the expression of known Lef1-dependent gene targets in the caudal hypothalamus is differentially affected in the two lines. We will first confirm these results, and then evaluate the stress response through physiological measurements and behavioral assays. Lastly, we will test whether the human SNP affects Lef1 activity in response to stress or Wnt signaling, using a CRISPR/Cas9-mediated knock-in approach.

1773F **Conserved autism genes regulate GABAergic neuron plasticity in adult male *C. elegans*** Kristi Zoga, Michael Hart University of Pennsylvania

Structural changes to neuronal circuits have the potential to alter behavior and increase memory storage in the brain. Remodeling of GABAergic neurons and resulting changes to excitatory/inhibitory balance has been implicated in neurodevelopmental and neuropsychiatric conditions characterized by behavioral changes, including autism. We previously discovered that the GABAergic neuron, DVB, undergoes experience- and activity-dependent structural plasticity in adult male *C. elegans*. DVB neuron remodeling is characterized by progressive neurite outgrowth and branching that modifies specific synaptic connections, alters a step of male mating behavior (spicule protraction), and is regulated by conserved autism genes *NRXN1*/*nrx-1* and *NLGN3*/*nlg-1*. Here we screened 20 conserved autism-associated genes for roles in DVB remodeling and plasticity. Using confocal imaging and manual tracing of the DVB neuron at day 3 of adulthood, we analyzed the morphologic parameters of neurite length and branching. We also applied a novel multi-modal computational framework, CAJAL, for analysis and integration of DVB single-cell morphologic data. Together these approaches identified novel genes with roles in DVB remodeling, including *chd-1/CHD1*, *daf-18/PTEN*, *gap-2/SYNGAP1*, *unc-44/ANK2,* and others. Additionally, we screened the same list of conserved autism genes for roles in DVB behavioral plasticity, which identified genes with roles in both spicule protraction behavior and DVB morphologic remodeling, and genes with specific roles in only behavior or morphologic remodeling. We are currently characterizing each candidate gene for expression, localization, and function within DVB and the spicule protraction circuit. Mutations in *unc-44/ANK2*, an ankyrin important for cytoskeleton organization and axon maintenance, increase DVB morphologic plasticity and number of synaptic puncta and decrease inhibition of spicule protraction. Characterization of *unc-44/ANK2* isoforms and expression suggests a potential cell non-autonomous role in suppression of morphologic plasticity in DVB. Mutations in *set*-4/*KMT5B*, a histone H4K20 methyltransferase, increase DVB morphologic plasticity without altering spicule protraction or synaptic architecture. Our candidate screening approach has provided mechanistic insight into genes that contribute to GABAergic morphologic and behavioral plasticity, including novel roles for conserved autism genes.

1774F **Novel insights from Humanization in *C. elegans*: Functional Rescue of G-alpha(o)-Dependent Behavior** Jacqueline S Cho, Adam Friedberg Neuroscience, Brown University

Sleep is an essential behavior observed across species. A key characteristic of sleep is behavioral quiescence which includes cessation of movement and feeding. *C. elegans* experience two states that can be classified as sleep: developmentally time sleep (DTS), which occurs just before molting during periods known as lethargus, and stress-induced sleep (SIS), which occurs following exposure to environmental stressors (Trojanowski & Raizen, 2015). A classical genetic screen identified genes capable of disrupting sleep in *C. elegans*, and *goa-1*, a gene that encodes a G-alpha(o), was identified (Huang 2017). Notably, variation in the chromosomal-region containing the human ortholog GNAO1 is associated with insomnia, suggesting GNAO1 may have a potential regulatory role in sleep across species (Lane 2019). To investigate functional conservation of G-alpha(o), we humanize *goa-1* in *C. elegans* using a two-phase approach: (1) replacing the 3' coding sequences of *goa-1* with the corresponding sequence of *GNAO1*, and then (2) replacing the 5' coding sequences of *goa-1* after confirming viability of step 1. The final construct retains the endogenous intron 2, the largest intronic region. We will assess conservation of G-alpha(o) function by assaying four *goa-1* dependent behaviors: reversal rate, egg-laying, DTS, and SIS. Through direct comparison of wild-type and humanized C. elegans, we aim to measure the rescue of G-alpha(o) function in sleep and non-sleep behaviors. Leveraging population-genetics and CRISPR-Cas9 technologies, we investigate novel contributions of G-protein signaling in conserved sleep mechanisms.

1775F **The glutamate receptor *glr-5* regulates stress-induced sleep in *C. elegans*** Caroline Kominick, Matthew Nelson St. Joseph›s University

The stress response of *C. elegans* consists of two distinct behavioral states: 1) Threat avoidance characterized by heightened activity; 2) stress-induced sleep which promotes recovery. These opposite and essential behaviors are controlled by a small number of neurons. Avoidance and arousal require sensory neurons like ADL and ASH, and the hub interneuron RMG. Stress-induced sleep requires the ALA and RIS interneurons. We showed that the *npr-38* neuropeptide receptor is required in the ADL neurons for both avoidance and sleep, suggesting that the ADL neurons also function during sleep. The ADL neurons express many neuropeptide genes and are also glutamatergic. To determine if glutamate signaling from the ADL neurons regulates sleep, we examined the glutamate receptor *glr-5*, which is expressed in cells postsynaptic to the ADL neurons, including RMG, ALA, RIM, AIB, RIS, and PVC. We used CRISPR to construct a loss-of-function strain (*glr-5(stj554)*) and obtained a deletion mutant (*glr-5(tm3506)*). Surprisingly, *glr-5(stj554)* animals displayed reduced stress-induced sleep while *glr-5(tm3506)* animals showed a significant increase in sleep. We are testing the hypothesis that *tm3506* is a gain-of-function allele. To determine where *glr-5* functions during sleep we are conducting cell-specific rescue experiments. So far, expression of *glr-5* in the RMG neuron does not restore sleep. The site-of-action is still unknown.

1776F **The neuropeptide receptor *npr-4* and the neuropeptide *nlp-61* regulate stress-induced sleep in *C. elegans*** Quinn M Howe, Matthew Nelson Biology, Saint Joseph›s University

Sleep is evolutionarily ancient and regulated by conserved signaling mechanisms. Recent work in our lab has identified the neuropeptide receptor *npr-38* as a key regulator of stress-induced sleep. We found that *npr-38* is required in the ADL neurons for the full induction of sleep. *npr-38* is also expressed in the ASH, RMG, AIB, ALA, and RIS neurons, which form a 6-celled circuit. We sought to better understand how the ADL neurons, as well as the other cells in this circuit, regulate sleep. My project focuses on characterizing the function of *nlp-61*, a neuropeptide expressed in the ADL neurons, and *npr-4* a receptor expressed in all 6 cells of this simple circuit. I have found that *nlp-61* is required for stress-induced sleep, while *npr-4* mutants display heightened sleep. Currently, I am using cell-specific rescue to determine the precise site of action for both genes.

1777F **Identification of transcription factors that bind to novel promoter motif in *C. elegans*** Nadia G Gaytan[1], Mailyn Nishiguchi[2], Sarah Hall[2] [1]Department of Biology, Program in Neuroscience, Syracuse University, [2]Syracuse University

Environmental stress in early life can alter gene expression through epigenetic mechanisms, resulting in phenotypic changes in adulthood. Using *C. elegans*, we have previously shown how developmental programming of neuronal genes can lead to behavioral plasticity. When larvae experience stressful conditions such as starvation and crowding, they develop into an alternate life stage known as dauer, which is stress resistant and non-aging. When the stressful conditions alleviate, the animals will exit dauer and develop into postdauer (PD) adults. In contrast, favorable conditions in early life promote continuous development of larvae until they grow into a control (CON) adult. We have shown previously that the *osm-9* TRPV channel gene, required for chemosensory and olfactory behaviors, is downregulated in ADL neurons of PD adults while remains expressed in CON adults. OSM-9 and the ADL neuron is required for the detection of the ascr#3 pheromone, which signals unfavorable crowding conditions at high concentrations and promotes avoidance behaviors in CON hermaphrodites. In contrast, PD hermaphrodites show a lack of ascr#3 avoidance. We showed previously that a conserved sequence in the upstream regulatory region of the *osm-9* gene is required for its downregulation in PD ADL neurons and is bound by DAF-3/SMAD in TGF-β signaling pathway. Interestingly, this sequence is found upstream of 1009 genes with expression patterns that are enriched for germline and neuronal-expressed genes.To identify additional pathways that may regulate gene expression through this conserved sequence, we performed a yeast one-hybrid screen of transcription factors with known expression in neurons. With an *osm-9*p::*gfp* reporter transgene, we have validated whether a subset of candidates identified in our screen plays a role in the developmental programming of *osm-9* in PD ADL neurons. Here, I present *osm-9* expression and ascr#3 avoidance results for three candidate transcription factors: NHR-64, SOMI-1, and EGL-43. Additionally, we have shown previously that TGF-β signaling in neurons can modulate germ cell proliferation by regulating the *lag-2* Notch ligand gene that contains a conserved motif in its upstream regulatory regions. We are currently utilizing auxin-induced degradation to investigate the tissue-specific role of DAF-3/SMAD in this process. Together, these experiments dissect the gene regulatory mechanisms by which early life stress affects phenotypic plasticity in *C. elegans*.

1778F **Drugging Worms to Study Substance Use Disorder** Shelby N Lauzon, Prince Mosely, HaoSheng Sun CDIB, The University of Alabama at Birmingham

Substance use disorder (SUD) or drug addiction is a ubiquitous problem affecting over thirty million people in America. Presently, few treatment options are available for SUD, despite our advances in SUD neurobiology. Most studies have primarily used rodents and humans as models, which do not allow for high-throughput screening of genetic contributors or potential therapeutic compounds. The benefits of using Caenorhabditis elegans (C.elegan) as a model are its short embryo to adult development, compact and well-delineated nervous system, and conserved neurobiological systems that underlie drug responses. We acutely and chronically expose worms to varying doses of stimulants and opioids to identify the behaviors associated with stimulants and opioids in C. elegans. Compared to control animals, the acute and chronic cocaine-exposed groups displayed a decrease in speed, amongst other locomotion parameters. We are currently investigating the neurotransmitter system(s) and molecular modulators that control cocaine-induced behavioral changes. Concurrently, we are currently generating molecular atlases of drug response nervous-system-wide in single neuron resolution using a combination of cell-type specific bulk analysis and single nuclei (sc) analysis of transcriptome and chromatin landscape. A major goal for future research is to establish a platform for high-throughput identification and characterization of novel regulators of drug-induced plasticity in distinct neuronal cell types, which can be used for clinical translation of improved therapeutics for SUD.

1779F **Investigating the cis-regulatory locus of the *ceh-43* homeobox gene in *Caenorhabditis elegans* neuronal specification** James Lao[1,2], Berta Vidal[1,2], Oliver Hobert[1,2] [1]Biological Sciences, Columbia University, [2]Howard Hughes Medical Institute

Terminal differentiation of neuron classes is regulated by homeodomain transcription factors, encoded by highly conserved homeobox genes across animal nervous systems. In *Caenorhabditis elegans*, each neuron class is defined by the expression of a unique combination of homeodomain proteins. Currently, 113 out of the 118 neuron classes of *C. elegans* are known to require a homeobox gene for proper terminal differentiation. Yet, there remain five neuron classes, including the ASJ sensory neuron pair, which do not have a known homeobox regulator. The Distalless/DLX ortholog *ceh-43* is expressed in ASJ

and several other neuron classes in the head, midbody, and tail (ADE, AIN, AIZ, ASJ, BDU, CAN, CEP, IL1, PDE, PVQ, SDQ). We previously showed that *ceh-43* is required for the differentiation of dopaminergic neurons and the ring interneuron AIZ, and we are now investigating potential additional roles of *ceh-43* in ASJ and other neurons. To circumvent the embryonic lethality of *ceh-43* null mutants, we generate novel cis-regulatory alleles of the *ceh-43* locus, seeking to selectively eliminate *ceh-43* expression in ASJ and/or other neuron classes in otherwise viable animals. We present here a cis-regulatory mutant analysis of *ceh-43* as a means of finding a homeobox regulator for ASJ, as well as a tool to better study *ceh-43* gene function in other *ceh-43*-positive neurons that have not yet been studied for an involvement of *ceh-43* (e.g. SDQ). Comprehensive analysis of homeobox function may point to an evolutionary and central role of this gene family in delineating neuronal identity specification during development.

1780F **Investigating aggregate feeding behavior across Caenorhabditis superfamily** Reina Eugene[1], Dustin Haskell[2], Michael Hart[3] [1]Liberal Arts, Pennsylvania State University, [2]Genetics, University Of Pennsylvania, [3]University Of Pennsylvania

Caenorhabditis elegans display two distinct foraging behaviors; solitary foraging or group foraging. These behavioral states depend on the integration of environmental cues including pheromones, food, touch, and oxygen/carbon dioxide levels. Aggregation of animals into groups during foraging is in part driven by Caenorhabditis elegans tendency to avoid higher oxygen levels in a lab setting. This also drives a related behavior termed bordering, where the animals feed on the border of the food lawn where bacteria is the thickest. Aggregation and bordering behavior is controlled by the conserved neuropeptide receptor npr-1/NPY gene, with mutations in the gene resulting in changes to aggregation behavior. While it is unclear if all nematode species aggregate, we hypothesize that this behavior and neuronal circuit may be conserved amongst members of the Caenorhabditis superfamily. To assay aggregation behavior, we pick 50 L4 (4th larval stage) animals of each species onto a 6-well plate filled with agar and seeded with a standard bacterial lawn (E. coli - OP50). Images of the plates are taken every 30 minutes on an automated WormWatcher platform and the number of aggregating worms (out of 50) are quantified at the 15-hour time point - day 1 adult animals. Loss of function npr-1 C. elegans display high levels of aggregation behavior and served as the controls. We find that Caenorhabditis aggregation behavior is not entirely conserved across species. In contrast, bordering behavior appears prevalent in all members of the clade tested thus far.

1781F **The SCRM-1 Phospholipid Scramblase Regulates Shedding of Extracellular Vesicles from the Ciliary Base** Alexis Semmel, Jessica Tanis University of Delaware

Small secreted extracellular vesicles (EVs) that contain bioactive molecules are shed by most, if not all, cell types. It is important to identify mechanisms of EV biogenesis in order to understand how this type of intercellular communication is regulated to impact physiological and pathological processes. EVs can be released from cilia, microtubule-based organelles that protrude from the cell surface. In our model organism Caenorhabditis elegans, EVs bud from the non-motile cilia of sensory neurons and are released into the environment through pores in the cuticle. We discovered that the CLHM-1 ion channel is a cargo in EVs released from the ciliary base, while the polycystin TRP channel is present in EVs shed from the cilium distal tip. Using C. elegans that express CLHM-1 tagged with tdTomato and PKD-2 tagged with GFP at single copy we are able to quantify shedding of the different EV subpopulations from the male tail RnB neurons. Phospholipid scramblases, including SCRM-1, are plasma membrane transporters of lipids, which randomly flip phospholipids between leaflets of the plasma membrane. Notably, activation of SCRM-1 has been shown to activate externalization of the phospholipid phosphatidylserine (PtdSer). Asymmetrical distribution of PtdSer is observed in platelet-derived microvesicles and apoptotic bodies. We sought to determine if loss of scrm-1, impacts EV biogenesis and sorting of the CLHM-1 and PKD-2 cargoes. Total internal reflection fluorescence (TIRF) microscopy was used to image EVs released from scrm-1 mutant and control animals expressing CLHM-1::tdTomato and PKD-2::GFP followed by Imaris spot detection to quantitate the number of EVs released into the environment. Deletion of scrm-1 caused a significant decrease in shedding of CLHM-1 EVs while the number of PKD-2 EVs was unaffected. Analysis of CLHM-1::tdTomato localization in the RnB neurons showed that the amount of CLHM-1 was reduced in the ciliary base, but no change was observed in the cilium proper. This suggests that regulation of PtdSer asymmetry in one ciliary subcompartments can be used to specifically regulate biogenesis of the EVs that are shed from that location.

1782F **Astrocyte Regulation of Excitatory Synapse Formation** Hallie A Youker[1], Nelson A Perez-Catalán[2], Katie Schneider[1], Jimmy A Kelly[2], Claire A Bui[2], Keiko A Hirono[2], Laurina A Manning[2], Chris Q Doe[2], Sarah D Ackerman[1] [1]Washington University in St. Louis, [2]University of Oregon

Vertebrate nervous system development is a complex process requiring tight regulation of the cellular processes required to form balanced neuronal circuitry. Astrocytes, the most abundant glial cell found in the central nervous system, is a key regulator of neural circuit formation. Astrocytes modify neural circuits by regulating synaptogenesis, synapse maintenance, and through direct synaptic pruning, modifying the number of synaptic connections in an activity-dependent manner. Astrocytes remain closely associated with synaptic terminals and mediate neuron-neuron communication via various extracellular signals,

both secreted and membrane-bound, monitoring the activity of these neurons and adjusting circuitry accordingly. Imbalanced neural circuits manifests various neurological disorders, from epilepsy to schizophrenia. The clinical relevance of astrocytes in development has led to closer examination of the ways in which astrocytes maintain healthy neural circuits. In fact, recent *in vitro* work has demonstrated a direct link by which astrocytes instruct the formation of glutamatergic synapses, a type of excitatory synapse. However, very little work has been done to explore the astrocytic mechanisms regulating other synapse types, especially *in vivo*. To address this, we used the power of *Drosophila* genetics to study the role of various astrocytic extracellular matrix (ECM) proteins in cholinergic synapse formation. Using *alrm-Gal4*, an astrocyte-specific promoter, we drove selected RNAi lines for the knockdown of various ECM components in astrocytes, and measured changes in cholinergic synapse density using Synaptic Tagging with Recombination. Excitingly, we identified a subunit of laminin in astrocytes that causes a significant reduction in cholinergic synapse density. This finding was only present in late-stage *Drosophila* larvae (L3), suggesting a potential role of this astrocyte-associated molecule in a pruning pathway, rather than in synaptogenesis. Importantly, we observe significant changes in synapse number without underlying differences in astrocyte development. Thus, we have identified an astrocyte-specific cue that drives formation of the most abundant excitatory synapse type in *Drosophila*. As laminin is a common extracellular ligand, future efforts will focus on identifying the neuron-bound receptor that responds to this astrocytic cue, and how astrocyte-neuron signaling triggers synapse instability.

1783F **Abnormal spindle employs non-cell autonomous mechanisms to promote proper brain growth and development** Shalini Chakraborty, Todd Schoborg University of Wyoming

Autosomal recessive primary microcephaly (MCPH) is a congenital neurodevelopmental disorder (NDD) characterized by a reduction in brain volume, intellectual disabilities, and lifespan. The most common cause of human MCPH is homozygous mutations in the *abnormal spindle like microcephaly associated* (*aspm*) gene, which has a *Drosophila* ortholog called *abnormal spindle (asp).* Although the MCPH phenotype is clinically well-characterized, the cellular and molecular mechanisms responsible for the small brain phenotype remain poorly understood. This is because we lack fundamental knowledge of Asp's neurodevelopmental behavior, the cell types in which it is expressed, and the molecular functions of Asp that dictate proper neurogenesis in time and space. To address this gap in knowledge, we first assessed the endogenous localization of Asp in various cell types of the fly CNS across development. Asp is highly expressed in all neurogenic cell types from embryonic to late larval stages, with low levels of protein persisting into pupal and adult neurons. Cell-type specific genetic rescue assays expressing either full length (Asp^{FL}) or a truncated rescue fragment (Asp^{MF}) in asymmetrically dividing central brain and optic lobe medulla neuroblasts (*Insc-Gal4*) was sufficient to restore central brain, but not optic lobe size and morphology in adults. We then further dissected the remaining lateral-most neural stem cell populations in the optic lobe and found that expression in the lamina precursor cells alone (*GCM-Gal4*) was sufficient to restore optic lobe size and neuropil organization along with the lamina in adult *asp* mutants. It also restored neuroepithelial cell and tissue morphology of the larval outer proliferation center (OPC) despite the fact that these cells did not express the rescue fragments, pointing towards a non-cell autonomous role for Asp in the developing optic lobe. Transcriptional profiling and protein interaction experiments highlighted Notch signaling and temporal transcription factors (tTFs) as potential downstream effectors of Asp's non-cell autonomous function, providing new insights into how Asp promotes proper brain growth and development that may be relevant in the etiology of human MCPH.

1784F **Natural variation in behavioral and transcriptional responses to social isolation in *Drosophila melanogaster*** Jesús E Sotelo-Fonseca[1], Chengcheng Du[1], Corbin Jones[2], Pelin Volkan[3] [1]Biology, Duke University, [2]Genetics, University of North Carolina at Chapel Hill, [3]Duke University

Social experience has long-lasting effects on animal behaviors. In humans, social isolation contributes to neuropsychiatric conditions like depression, anxiety, aggression, and sleep disruptions. Responses to social isolation have been observed in other animals ranging from mice to flies. Nevertheless, effects of isolation vary among individuals, and how these are shaped by environmental and genetic constraints remains unclear. *Drosophila melanogaster* is an ideal system to address these questions. In addition to being an excellent genetic model, *D. melanogaster* is a human commensal species, and its demographic history mirrors that of humans. For this reason, geographically diverse populations of *D. melanogaster* serve as a natural experiment to investigate the impact of genetic variation on behavioral responses to isolation. We specifically focus on the social experience-dependent changes in male courtship behaviors as 1) previous research has shown that social isolation increases courtship behavior in *D. melanogaster* laboratory strains, 2) genes and neuronal circuits that control male courtship have been delineated, and 3) previous studies in our lab have found courtship regulatory genes that are differentially expressed between grouped and isolated laboratory strain flies.

We have identified variation in courtship responses to social isolation in six *D. melanogaster* Global Diversity Lines collected from five different continents. Some of these lines exhibited contrasting courtship responses between grouped and isolated flies. We hypothesize that differential gene expression in the male brains across populations underlies differences in courtship

behavioral responses to the social environment. To test this hypothesis, we performed bulk RNA-seq from the brains of isolated or grouped male flies in specific GDLs. Comparison of line-specific gene expression responses to social isolation identified a small number of candidate genes. We anticipate future experiments to causally link the gene expression to behavioral changes will inform how natural genetic variation influences specific pathways to modulate susceptibility to social isolation.

1785F **Efficient methods for expanding cell-type-specific genetic tools for studying neuronal development in the Drosophila visual system** Elizabeth Abraham[1], Yu-Chieh David Chen[2], Claude Desplan[2] [1]New York University, [2]Developmental Biology, New York University

Drosophila has emerged as a powerful model for unveiling fundamental principles underlying neuronal development. Currently, we have a comprehensive understanding of the patterning mechanisms driving cell differentiation in the *Drosophila* visual system. However, there is still a significant knowledge gap concerning how each cell type acquires its unique neuronal structure and establishes precise connections during development. To address these developmental questions, the first crucial task is the precise identification and labeling of every neuronal cell type. The Desplan Lab has recently established a pipeline to identify two genes that are specifically expressed in one cell type based on the single-cell transcriptomic atlas in the developing *Drosophila* optic lobe. We can selectively label and distinguish specific cell types through the generation of split-GAL4 of the selected gene pairs. Despite the successful identification of distinct cells through this method, efficient gene-specific split-GAL4 generation is limited to certain marker genes that contain transposable elements in the coding intronic regions. Additionally, there are some cell types that lack optimal gene pairs for gene-specific split-GAL4 genetic intersection. Thus, there is a necessity for exploring alternative methods to allow for the efficient generation of highly cell-type-specific genetic tools for optic lobe neurons, including utilization of the attP/attB integrase system and analysis of multi-omic data. In my research, I aim to extend the genetic tool kits for studying all cell types in the developing optic lobes by 1) establishing a robust pipeline to use existing single attP knock-in fly line in Drosophila to make gene-specific split-GAL4 lines via recombinase-mediated cassette exchange (RMCE); 2) selecting enhancers from our recent multi-omic dataset (scRNAseq + scATACseq) for generating split-GAL4 lines for the cell types without ideal gene pairs. Our primary aim is to expand our genetic toolkit for pinpointing uncharacterized cell types, thus overcoming current genetic constraints. These advanced tools will facilitate a comprehensive exploration of neuronal development within distinct cell types in *Drosophila* optic lobes, with potential applications extending to other neural and non-neural tissues of the fly.

1786F **Effect of social experience on gene expression, circuit function and behaviors** Chengcheng C. Du[1], Jesus E.S. Fonseca[1], Shania C. Appadoo[1], Luis C. Garcia[1], Yuta Mabuchi[2], Nilay Yapici[3], Corbin Jones[4], Pelin C. Volkan[5] [1]Department of Biology, Duke University, [2]Columbia University, [3]Cornell University, [4]UNC-CH, [5]Biology, Department of Biology, Duke University

Social experiences influence various animal behaviors, but the molecular and circuit-based mechanisms underlying experience-dependent behavioral changes are not known. Recent evidence suggests a link between experience-induced alterations in gene regulation and behavioral adjustments. The Drosophila courtship behavior serves as an excellent model for investigating this relationship, where connections between courtship behaviors, genes, and circuits have been elucidated. In Drosophila melanogaster, Fruitless^{M} (Fru^{M}) and Doublesex^{M} (Dsx^{M}) are master transcription factors controlling sex-specific innate and learned courtship behaviors. *fru^{M}* is expressed in around 2000 interconnected neurons throughout the nervous system, marking courtship circuits, while *dsx^{M}* is predominantly expressed in approximately 700 neurons and mainly found in non-neuronal cells. Social isolation boosts courtship vigor in wild-type males, while monosexual grouping suppresses it. We previously showed that *dsx* and *fru* levels are modified with social experience and pheromone receptor signaling in the antennae where pheromone-sensing olfactory neurons reside. These lead to the misregulation of *fru* and *dsx* target genes and pheromone responses. In the central courtship circuits, social isolation also increases the baseline and evoked neuronal activity. Single-cell RNA sequencing from *fru* and *dsx*-positive neurons in grouped and isolated male brains revealed that social isolation affects *fru* expression in only a subset of neurons within the courtship circuit. Whole-brain transcriptome profiles identified an upregulation of *dsx* in isolated male brains. Pheromone-sensing Or47b olfactory circuit contributes to the suppression of courtship by grouped males. Interestingly, blocking the activity of different pheromone circuits results in distinct effects on gene regulation in the brain. This suggests different social signals in the environment trigger different transcriptional responses in the brain. We particularly find neuropeptides and their receptors associated with the modulation of behaviors are differentially regulated by different pheromone circuit activity. Our findings offer insights into the fundamental mechanisms through which sensory experiences drive behavioral adjustments by altering the expression of genes critical for neural circuit structure and function.

1787F **Temporal manipulation of serotonin during development creates persistent behavioral defects in *Drosophila* larvae** Hayden Schneider[1], Ken'Nisha Patton[1], Emma Walker[1], Will Dugan[1], Douglas H Roossien[2] [1]Ball State University, [2]Biology, Ball State University

The neurotransmitter serotonin modulates a variety of behaviors in *Drosophila melanogaster*, such as locomotion, feeding, courtship, and aggression. Prior to assuming this canonical role, however, serotonin acts as an autocrine signaling molecule regulating the development of its own axons. For example, mutations limiting serotonin production cause an increase in axon outgrowth and branching, whereas exogenous serotonin decreases outgrowth and branching in cultured serotonergic neurons. In vertebrates, there is a critical period during development where serotonin signaling can cause these structural changes, but how this impacts circuit function and behavior at later stages of development is unclear. Here we performed pharmacological manipulations of serotonin temporally during embryonic and first instar larval stages, the period in which robust serotonergic axon outgrowth and wiring occurs, after which serotonin levels were allowed to return to normal during second and third instar stages. We then assayed behavior at the late third instar stage, and found both locomotion and feeding to be disrupted. Defects in these behaviors were equivalent regardless of whether serotonin was increased or decreased. This suggests that dysregulation of serotonin levels during this critical period of serotonergic axon development caused defects in the circuits underlying these behaviors, which persisted into later stages of development.

1788F **Ecdysis Triggering Hormone Both Activates and Suppresses Ecdysis Motor Programs** Niall Dermady[1], Richard Farrel III[2], Haojiang Luan[3], Benjamin White[3] [1]NIM, National Institutes of Health, [2]National Institutes of Health, [3]NIMH, National Institutes of Health

Insects must shed their exoskeletons during development in order to grow. This process is called ecdysis and is initiated by the release of Ecdysis Triggering Hormone (ETH). In *Drosophila*, ETH is known to coordinate the motor programs governing ecdysis by positively regulating neurons that express the A isoform of the ETH receptor, ETHRA. A second isoform of the ETH receptor, ETHRB, is also expressed in a large number of *Drosophila* neurons, but the role of these neurons in ecdysis has been less well characterized. Here, we use an ETHRB-Gal4 driver to selectively activate or inhibit ETHRB-expressing neurons at the time of adult ecdysis using optogenetic and thermogenetic techniques.

Adult ecdysis involves the serial execution of two principal motor programs, the first is used to exit the pupal case at eclosion and the second is used to subsequently expand the wings. We find that selectively activating ETHRB neurons using UAS-dTrpA1 robustly suppresses eclosion. Preliminary data also indicates that suppressing ETHRB neurons using UAS-GtACR1 accelerates eclosion. Similarly, suppressing ETHRB-expressing neurons using UAS-GtACR1 significantly accelerates wing expansion, while activating them using UAS-dTrpA1 suppresses this motor program. Surprisingly, our results thus indicate that ETHRB-expressing neurons—in contrast to neurons expressing ETHRA—inhibit the adult ecdysis motor programs in *Drosophila*. This supports a proposed model in which ETHRB neurons act as “first responders” to ETH and provide blanket inhibition to the ecdysis neural network by targeting the ETHRA neurons responsible for driving the ecdysis motor programs. In this model, the sequential suppression of subsets of ETHRB neurons relieves inhibition first of the ETHRA neurons that drive eclosion and then of the ETHRA neurons that drive wing expansion. A major goal of future work is to test this model by identifying the population(s) of ETHRB neurons that inhibit wing expansion and eclosion and to determine whether the ETHRA neurons regulating these motor programs are among their direct targets.

1789F **A multi-input optic glomerulus mediates diverse behavioral responses to visual objects.** Ines M.A. Ribeiro[1,2], Maria Leonte[2], Michael Sauter[2], Stephan Prech[2], Alexander Borst[2] [1]LMU-Munich, [2]Max Planck Institute for Biological Intelligence

Drosophila melanogaster males and females perform distinct visually guided behaviors during courtship. Upon exposure to female non-volatile pheromones, the male orients towards and chases the female to remain in close proximity to her and beat competition. In contrast, the female turns away from the male, seemingly exploring the courtship arena. Both females and non-courting males turn away from another fruit fly with undefined chemosensory profile. Vision starts as an abstract 2-dimensional array of light intensities fluctuating over time, from which visual features, such as discrete objects or color, are extracted. Visual projection neurons then relay varied combinations of visual features to the central brain. During courtship, Drosophila males rely on LC10a visual projection neurons to track the female. LC10a are part of the LC10-group neurons, that co-project to the anterior optic tubercle (AOTU). The projection of different visual neuron types to the same retinorecipient brain region could underlie convergence of sets of features to create a rich representation of a visual object. Alternatively, the different LC10-group neuron types could form parallel output neural circuits that use the spatial organization present in the AOTU for downstream operations. To discern between these possibilities, we used single-pair and fly-on-ball behavioral assays, functional imaging and optogenetics. We found that LC10d neurons mediate avoidance of a fly without a defined chemosensory signature in non-courting males and females, and of a small visual object in the fly-on-ball assay. Functional imaging of neuronal responses to a barrage of visual features revealed that both LC10a and LC10d neurons detect discrete objects, with LC10d neurons exhibiting overall broader tuning properties than LC10a. Activation of unilateral subsets of LC10d neurons, using the light-gated LOV-LexA genetic tool, elicits a small contralateral turn, whereas activation of similar numbers of LC10a cells leads to ipsilateral turns. LC10d and LC10a neurons similarly sense discrete objects but mediate opposing behaviors, implying that opposite valences of visual objects are encoded by LC10a (orienting towards) and LC10d (turning

away). The AOTU thus appears to function as a hub for processing visual cues in parallel, likely reliant on spatial organization, that subserve different, even opposing behaviors.

1790F ***Drosophila* neuronal Glucose-6-Phosphatase regulates glycogen storages via FMRFa signaling in the jump muscle** Tetsuya Miyamoto, Sheida Hedjazi, Chika Miyamoto, Hubert Amrein Cell Biology and Genetics, Texas A&M University

Neuropeptides and their cognate receptors are critical molecular effectors of diverse physiological processes, many of which modulate a variety of behaviors. We recently reported of a non-canonical function of the *Drosophila Glucose-6-Phosphatase* (*G6P*) gene in a small subset of neurosecretory cells in the CNS and showed that *G6P* mutant flies failed to maintain whole body glucose homeostasis, a phenotype that could be rescued by constitutive activation of *G6P* neurons. We proposed that *G6P* is required for adequate signaling in a subset of peptidergic neurons.

Here, we show that the *G6P* expressing neurons define 7 different groups of peptidergic neurons, 5 in the brain and 2 in the thoracic ganglia, expressing five different neuropeptides. One such group, located in the thoracic ganglia and expressing FMRFa neuropeptides, is necessary and sufficient to maintain whole body glucose homeostasis in starved flies. Moreover, we find that FMRFa neurons lacking *G6P* exhibit a smaller Golgi apparatus and have a reduced neuropeptide release capacity. Using *FMRFa Receptor$^{2A\text{-}GAL4}$* knock-in fly strain, we show that the *FMRFaR* gene is highly expressed in the jump muscle, with some weaker expression in the CNS. Muscle, and to a lower extent the fat body, is known to maintain large amounts of glycogen as a source for the mobilization of energy. We found that *G6P*/*FMRFa*/*FMRFaR* mutant flies exhibited significantly reduced glycogen content not only in the jump muscle but also in the flight muscle and fat body when compared to wild-type flies. Taken together, our data suggest that *Drosophila* G6P is required to build up glycogen stores via FMRFa signaling, primarily in the jump muscle and secondarily in other organs known to store glycogen.

1791F **Neuropeptide mediated changes in synaptic output at individual active zones** Stephen Clifford, Kiel G Ormerod Biology, Middle Tennessee State University

Drosophila melanogaster has become a popular model organism for the study of human neurological disorders due to its unparalleled versatility regarding genetic manipulation, and its extensive genomic conservation between flies and humans. Most human neurodegenerative disorders are attributable to mutations and alterations at the molecular level. Thus, establishing models to investigate the function of the cells that make up the nervous system, in neurons, is invaluable. Our laboratory uses a model glutamatergic synapse in *Drosophila* to investigate the roles of neuromodulators and dense core vesicular trafficking on nervous system health and function. To further investigate these roles, we aimed to establish a functioning GCaMP assay using GCaMP8 ultra-fast high-resolution fluorescence microscopy, allowing us a higher resolution of optical neurotransmitter fusion dynamics than other current imaging techniques readily available to us. Many neuropeptides are known to modify the output of synapses by altering pre and postsynaptic machinery. Here we exploit GGaMP imaging to investigate if different unique neuropeptides alter release of SVs at active zone resolution. We additionally use genetic approaches to alter putative proteins involved in this neuropeptide-mediated change in synaptic output to further elucidate these molecular and cellular processes. Abnormalities in the packaging or release of these molecules results in some of society's most severe and debilitating diseases and conditions including neurological disorders, cancers, obesity, diabetes, and many other conditions. However, before we can begin to understand and treat devastating diseases and conditions that affect our society, we must understand the basic components of cellular communication and what happens when they malfunction or breakdown.

1792F **5-HT1A regulates axon outgrowth in a subpopulation of *Drosophila* serotonergic neurons** Ava Kinser[1], Delaney Long[1], Abby Olalde-Welling[1], Luke Brewer[1], Juri Lim[1], Dayle Matheny[1], Breanna Long[1], Douglas H Roossien[2] [1]Ball State University, [2]Biology, Ball State University

Serotonergic neurons produce extensively branched axons that fill most of the central nervous system, where they modulate a wide variety of behaviors. Proper behavioral output therefore depends on the precise outgrowth and targeting of serotonergic axons during development. To direct outgrowth, serotonergic neurons utilize serotonin as a signaling molecule prior to it assuming its neurotransmitter role. This process, termed serotonin autoregulation, regulates axon outgrowth, branching, and varicosity development of serotonergic neurons. However, the receptor that mediates serotonin autoregulation is unknown. Serotonin receptors are expressed either in non-serotonin producing neurons as heteroreceptors or in serotonergic neurons as autoreceptors. In non-serotonin producing neurons, serotonin receptor activity regulates neurite outgrowth and branching. Yet, there has not yet been a systematic test of the role of autoreceptor activity in the outgrowth of serotonergic axons. Here we asked if serotonin receptor 5-HT1A plays a role in serotonergic axon outgrowth and branching. Using cultured *Drosophila* serotonergic neurons, we found that exogenous serotonin reduced axon length and branching only in those expressing 5-HT1A. Pharmacological activation of 5-HT1A led to reduced axon length and branching, whereas disruption

of 5-HT1A rescued outgrowth in the presence of exogenous serotonin. Altogether this suggests 5-HT1A is a serotonin autoreceptor in a subpopulation of serotonergic neurons and initiates signaling pathways that regulate axon outgrowth and branching during *Drosophila* development.

1793F **Serotonin autoreceptors are differentially and dynamically expressed throughout development in *Drosophila*** Luke Brewer, Juri Lim, Solmari Solano, Douglas H Roossien Biology, Ball State University

Serotonin receptors (5-HTRs) are G-protein couple receptors expressed throughout the nervous system, where they are responsible for modulating a variety of behaviors such as mood, sleep, and appetite. Serotonin receptors are expressed either in non-serotonin producing neurons (heteroreceptors) or in serotonergic neurons (autoreceptors), and can have divergent function depending on which. In addition, the intracellular signaling pathways initiated by the same serotonin receptor can diverge depending on whether they are acting as hetero- or autoreceptors. We recently found that 5-HT1A is expressed in a subpopulation of cultured *Drosophila* serotonergic neurons. This is in agreement with reports by others suggesting 5-HT1A and 5-HT1B are autoreceptors in the adult *Drosophila* brain, while conflicting with a report suggesting there are no autoreceptors in the third instar larval brain. We therefore performed a systematic study profiling the spatial and temporal expression of all five *Drosophila* serotonin receptors during the larval and adult stages. Because there are no viable antibodies toward *Drosophila* serotonin receptors, we used a genetic labeling approach. Flies from either 5-HTR-Gal4 or 5-HTR-LexA drivers were crossed to fluorescent reporter lines, and brains from offspring were stained with anti-5HT. Co-labeled serotonergic neurons were scored and mapped to previously published anatomical maps of larval or adult serotonergic neurons. We found that each serotonin receptor is expressed in subpopulations of serotonergic neurons, with some overlap. In general, the number of serotonergic neurons is highest in first instar stages followed by a decrease over time. This suggests serotonergic neurons consist of subpopulations with diverse and dynamic gene expression programs during early *Drosophila* development.

1794F **Neuropeptide Biology in Drosophila** Hardik Bansal, Veronika g Mousa, Tadros a Hana, Kiel G Ormerod Biology, Middle Tennessee State University

Neuromodulatory substances regulate critical processes spanning from regulated secretion to physiology and behavior. Consequently, most genomes encode hundreds of neuromodulatory substances and their receptors. Within the Drosophila genome over 30 genes encode for one of the major classes of neuromodulators in neuropeptides, and an even greater number encode for neuropeptide receptors. Neuropeptides are packaged within cells in large electron dense structures known as dense core vesicles (DCVs). DCVs are known to transport, store, and release neuropeptides and proteins at multiple cellular locations, which mediate critical biological processes like synaptogenesis, synaptic transmission, synaptic plasticity, and others. However, much of the cellular machinery involved in sorting, processing, trafficking, and releasing DCV content remains largely unknown. Here we have taken advantage of the genetic and molecular toolkits of Drosophila to address these fundamental questions about DCV biology. We have identified several novel roles for motor proteins and their subunits in trafficking and delivering DCVs to synapses. We have also identified several critical resident DCV proteins that are necessary for proper processing of prepropeptides into bioactive peptides ultimately impacting their ability to be trafficked to and undergo regulated secretion from the neuromuscular junction. Additionally, we have created over 20 different fluorescently tagged neuropeptide and resident neuropeptide proteins to characterize the cellular mechanisms of sorting of uniquely labeled cargo. Lastly, using quantal level imaging, we are characterizing the synaptic machinery mediating trafficking and release of DCVs. Our results create novel tools and provide important insights into the proteins and pathways regulating DCV sorting, trafficking, and secretion in vivo.

1795F **The RNA binding protein Nab2 regulates m^{6}A levels and splicing of the RhoGEF *trio* transcript to govern axon development** Carly Lancaster[1], Pranav Yalamanchili[1], Sara W Leung[2], Matthew Tegowski[3], Kate Meyer[3], Anita Corbett[1], Ken Moberg[1] [1]Emory University, [2]Biology, Emory University, [3]Neurobiology, Duke University

The evolutionarily conserved zinc finger polyadenosine RNA binding protein Nab2/ZC3H14 is implicated in several post transcriptional regulatory processes including polyadenylation, 3' end formation, splicing, and nuclear export. Mutations in the human *ZC3H14* gene cause a form of nonsyndromic intellectual disability, indicating that ZC3H14 is essential for proper brain function. To explore the function of Nab2/ZC3H14 in neurons, we performed RNA-Sequencing analysis of neuronally-enriched tissues from *Nab2* mutant flies. This analysis revealed that a small number of neuronally enriched transcripts show aberrant splicing or intron retention upon loss of Nab2. Our subsequent studies of one of these transcripts, the sex-determining factor *Sxl*, using DART (deamination adjacent to RNA modification targets) and meRIP-qPCR have uncovered a novel role for Nab2 in modulating levels of N^6-methyladenosine (m^{6}A) in a *Sxl* exon and intron. Prior work demonstrating that Nab2 is required within neurons is consistent with the observation that patients with loss of function mutations in *ZC3H14* suffer from intellectual disability. These data led us to focus on brain mRNAs that are regulated by Nab2/ZC3H14. One of these is a well-

conserved growth cone guidance factor, the guanine-nucleotide exchange factor (GEF) Trio, whose vertebrate homolog TRIO acts through the F-actin regulatory GTPases Rho and Rac to guide axon projection. We find that Nab2 controls Trio levels in the fly brain by modulating an intron-retention event within the 5' UTR of *trio* mRNA isoforms, and this mechanism appears to be dependent on m^6A levels within the *trio* pre-mRNA. Data will be presented on the role of the m^6A methyltransferase Mettl3 and Nab2 in controlling *trio* splicing and expression, and downstream effects of Nab2 loss on Trio protein and GEF activity in regulating axon development within the *Drosophila* central nervous system. Together, these studies provide possible insight into how the regulation of m^6A methylation by a conserved RNA binding protein is critical for proper neuronal function.

1796F **Neuronal interaction mediated by Dscam1 receptor differentially guides dendrite formation and axon targeting in motoneuron 24** Kathy Bui, Daichi Kamiyama Cell Biology, University of Georgia

Necessary for functional neural connections are subcellular structures that send out and receive neurotransmitter signals (e.g., axon terminals and dendrites, respectively). Previously, our group showed that dendrite formation occurs precisely at the interaction site between *Drosophila* aCC motoneuron and MP1 neuron – mediated by Down syndrome adhesion molecule (Dscam1) on the membrane. However, it is known that dendrites of 36 motoneurons form at unique sites – thus, an ensuing question: does the Dscam1 receptor play a role at other sites where motoneuron dendrites form and if so, how? Using established techniques of genetics, retrograde labeling, and confocal microscopy, we address whether neuronal interaction, mediated by Dscam1 receptor, plays a broad role in dendrite formation among motoneurons. In this study, we show that the Dscam1 receptor also can play a role in motoneuron 24 (MN24) dendrite formation in the *Drosophila* embryonic central nervous system due to its distinct location from the aCC-MP1 contact site, its highly stereotyped dendrite development, and the availability of genetic tools that allow for targeted cell-specific knockdown. Using retrograde dye labeling, we label single MN24 and show a significant reduction in dendrite branches that form in the *dscam1* mutant background. Unexpectedly, we also show a severe defect in axon targeting of MN24, suggesting that Dscam1 plays multiple roles for neurite development in MN24. To determine whether Dscam1 plays a cell-autonomous function, we use MN24-specific expression of short hairpin RNA for *dscam1* knockdown and show a similar reduction in dendrite branches (but a milder axon targeting defect) compared to that in the *dscam1* mutant. To determine whether Dscam1 plays a non-cell-autonomous function, we use *dscam1* knockdown in a subset of neuronal projections overlapping with the site of MN24 dendrite formation and, surprisingly, phenocopies the reduction in dendrite branches and the severe axon targeting defect. Altogether, this study highlights the differential roles of the Dscam1 receptor for dendrite formation and axon targeting within a single motoneuron. The sets of experiments suggest that Dscam1-Dscam1 interaction regulates dendrite formation whereas Dscam1 interaction with another ligand regulates axon targeting in MN24. Overall, we conclude that Dscam1 mediates diverse functions within a single neuron, refining the current understanding of Dscam1 function for neural circuitry formation.

1797F **Neuropile Ensheathing Glia Modulate Seizure Susceptibility of *Drosophila melanogaster*** Lexis Grandel, Samantha Casciani, Dionna DeFazio, Mar Hinestroza, Sarah Iannone, Alexis Hill Biology, College of the Holy Cross

The nervous system must be able to respond quickly and appropriately to changes in environmental conditions, such as exposure to extreme temperatures. Homeostatic control mechanisms that mediate such responses are essential for survival, allowing behavioral and other bodily functions to remain intact under environmental pressure. Here we show that in *Drosophila melanogaster*, a specific type of glial cell, neuropile ensheathing glia (EGN), plays a significant role in regulating the nervous system. Genetic manipulations specifically in EGN affect susceptibility to heat-induced and bang-induced seizure assays in the adult fly. In other contexts, EGN have been shown to have phagocytic functions. Therefore, we hypothesized that the phagocytic function of EGN may underlie its role in mediating seizure susceptibility. Supporting this hypothesis, EGN-specific knockdown of the engulfment receptor *draper* increases seizure susceptibility. We have also determined that EGN-specific knockdown of the voltage-gated potassium channel gene *seizure* (*sei*) and the sodium/potassium/chloride transporter *ncc69* increase seizure susceptibility, though whether these genes affect phagocytic functions of EGN has not yet been tested. Additional ongoing and future experiments will determine whether developmental and/or adult expression of EGN *draper*, *sei*, and *ncc69* modulate adult neural excitability and behavior in seizure susceptibility assays. This project contributes to our understanding of the important roles that glia play in modulating homeostatic nervous system control. Furthermore, we specifically identify EGN as a glial subtype that may allow us to discover key genes and mechanisms by which glia regulate nervous system activity and behavior.

1798F **Alterations in Sequential Motor Patterns of a Model of Neurofibromatosis Type 1** Hannah Brunner[1], Genesis Omana-Suarez[1,2], Seth Tomchik[1,2,3] [1]Neuroscience and Pharmacology, University of Iowa, [2]Scripps University, [3]Pediatrics, University of Iowa

Neurofibromatosis type 1 is a genetic disorder caused by the loss of the neurofibromin protein (Nf1). Neurofibromatosis has a wide range of physical and cognitive manifestations including autism spectrum disorder and attention deficit hyperactivity

disorder. Loss of Nf1 function may alter circuit activity, leading to behavior changes. We have found that loss of Nf1 in *Drosophila* alters spontaneous motor behaviors, including increasing grooming frequency. Whether Nf1 deficiency alters the frequency and pattern of sensory-evoked behaviors is unknown. If flies are covered with dust, they engage in vigorous grooming to remove the dust. Here we test the effects of dusting wild-type and *nf1* mutant flies, and if the typical grooming hierarchy has been affected. The data suggest that dusting increases grooming frequency in both genotypes, and that the grooming pattern in *nf1* mutants is altered: the normal sequence/prioritization is scrambled. This suggests that Nf1 loss both increases spontaneous grooming frequency and changes the pattern of this temporally-sequenced motor behavior.

1799F **Natural Variation in Neural Stem Cell Reactivation Offers Opportunity for Novel Insight into Neurogenesis** Taylor L. Nystrom, Alan O Bergland, Sarah Siegrist Biology, University of Virginia

During early life neurogenesis, neural stem cells (NSCs) give rise to thousands of morphologically and functionally diverse neurons that will build the adult brain. Ultimately, these neurons form functional circuits that allow proper functioning and survival of the adult animal. As neurogenesis progresses, NSCs transition through periods of quiescence and proliferation, with the transition from quiescence to proliferation referred to as reactivation. Reactivation is a temporally-regulated process that is known to be influenced by both intrinsic and extrinsic signaling, yet we still have a limited understanding of the genes and pathways involved in this process. Our work seeks to reveal new insights into the integration of intrinsic and extrinsic signaling by studying how an extrinsic factor, nutrient signaling, and an intrinsic factor, natural genetic variation, interact during NSC reactivation in Drosophila melanogaster. In order to capture genetic variation reflective of wild populations, we used a collection of wild-caught, geographically diverse, inbred lines of flies for all experiments. We then used classic developmental experiments to measure development and reactivation at distinct time points. Our work revealed significant differences in the timing of NSC reactivation from quiescence when genetically distinct larvae are raised in a common, calorically rich environment. We also saw that a delay in reactivation is generally correlated with a delay in overall developmental timing in the lines that we tested. Finally, in vitro experiments revealed a loss of variation in reactivation, suggesting that this variation is the result of a tissue extrinsic factor. These findings reveal that there is natural variation in NSC reactivation, as well as suggest a genetically encoded basis for variation in neurogenesis. Together, this knowledge provides a new opportunity to uncover novel genes or pathways involved in the transition from quiescence to proliferation.

1800F **Seizure and Motor Phenotypes Caused by Overexpression of *dube3a* in Neuronal and Glial Tissues** Megan N Sleep[1], Andrew Lacoste[1], Selene Tan[1], Lawrence T Reiter[2], Atulya Iyengar[1] [1]The University of Alabama, [2]The University of Tennessee Health Science Center

Duplication 15q (Dup15q) syndrome is thought to be caused primarily by increased copy number for the E3 ubiquitin ligase gene, *UBE3A*. Dup15q syndrome is characterized by motor deficits, developmental delays, and pharmacoresistant epilepsy. However, neuronal overexpression of *Ube3a* in vertebrate models of Dup15q syndrome fail to recapitulate seizure phenotypes. Interestingly, *Drosophila* overexpressing *dube3a* (the fly ortholog of *UBE3A*) in glial cells results in mechanical shock-induced seizures ("bang-sensitivity"). Here, we aimed to directly compare the motor phenotypes arising from *dube3a* overexpression in glia versus neurons in *Drosophila*. Specifically, we crossed UAS-*dube3a* with the glial driver, *repo*-GAL4, and the neuronal driver, *nsyb*-GAL4, and monitored open field behavioral phenotypes on a temperature-controlled stage. We used IowaFLI Tracker to quantify locomotion features such as average velocity, distance traveled, and time of activity. Flies overexpressing *dube3a* in glia (*repo* > *dube3a*) were significantly ataxic and experienced a 54% decline in average velocity compared to controls. In contrast, neuronal *dube3a* overexpression (*nsyb* > *dube3a*) had less effect on locomotion with a 24% decrease of average velocity (rank-sum test, *repo>dube3a* vs *nsyb>dube3a*, $p=3.4 \times 10^{-5}$). Interestingly, we observed several bouts of prolonged immobilization in *repo* > *dube3*a flies, indicative of underlying seizure activity. We found high temperature-stress (36-39 °C) also triggered seizure-related activity in *repo* > *dube3a* flies: caroming, spinning, uncontrolled wing buzzing, and immobilization. 48% of *repo* > *dube3a* flies experienced prolonged immobilization compared to 22% of *nsyb* > *dube3a* (χ^2 test, p=0.000054). Our findings confirm that glial overexpression, but not neuronal expression, of *dube3a* primarily contributes to the emergence of seizure-related phenotypes in the context of Dup15q syndrome.

1801F **Restoring histone acetylation homeostasis in Alzheimer's Disease using Tip60 HAT activators** Aprem Zaya[1], Akanksha Bhatnagar[1], Gu Gu Nge[1], Sandhya Kortagere[2], Felice Elefant[1] [1]Biology, Drexel University, [2]Microbiology and Immunology, Drexel University College of Medicine

Reduced histone acetylation in the brain caused by disruption of histone acetylation homeostasis is an early event in Alzheimer's Disease (AD) etiology that causes cognitive impairment prior to amyloid beta plaque formation. While genetics plays a role in the development of AD, over 90% of patients hospitalized for AD are diagnosed with sporadic AD, highlighting the importance of epigenetic dysregulation in disease progression. Numerous small molecules, including epigenetic modulators, are currently in clinical trials for AD. These epigenetics-based treatments aim to restore histone acetylation homeostasis by inhibiting histone deacetylases (HDACs), which remove acetyl marks from histones causing chromatin to

condense, leading to a reduction in gene expression. While promising, many HDAC inhibiting treatments are not specific to one HDAC, causing side effects likely due to global hyperacetylation. Our work focuses on Tip60 histone acetyltransferase (HAT), which generates pattern-specific acetyl marks onto histones to decondense chromatin, leading to enhanced transcription. We have shown that genetically increasing Tip60 HAT levels functionally rescues both cognitive deficits and expression of critical neuroplasticity genes repressed in a well-characterized Drosophila amyloid precursor protein (APP) model of AD by restoring histone acetylation homeostasis. Since our findings support a neuroprotective role for Tip60 HAT in AD, we propose an epigenetics-based therapeutic strategy using small molecules that selectively activate Tip60 HAT to restore histone acetylation homeostasis. We developed small molecule compounds using a structure based approach with a general HAT activator, then further optimized the compounds using a pharmacophore function-based approach. Using an in vitro HAT assay, we identified three of the compounds as robust and specific Tip60 HAT activators. Future work will elucidate the efficacy of these compounds in functional assays to assess locomotion and learning & memory in the Drosophila AD model and assess expression profiles of neuroplasticity genes responsible for cognitive function using real-time quantitative polymerase chain reaction (RT-qPCR). Our therapeutic strategy of designing a specific Tip60 HAT activator that restores histone acetylation homeostasis is a promising AD treatment that could address cognitive impairment that precedes other disease characteristics observed at later stages.

1802F **The genetic basis of neural circuit evolution for *Drosophila* mate preferences** Emily L Behrman, David L Stern HHMI Janelia Research Campus

Behaviors that arbitrate interspecies courtship are at the crux of evolutionary barriers between species. Species- and sex-specific pheromone profiles in *Drosophila* have rapidly diversified that signify species' identity to encourage courtship among conspecifics and discourage interspecies courtship. The dominant pheromone produced by *Drosophila melanogaster* females is an aphrodisiac to their males, but the same chemical cue inhibits courtship in male *D. simulans*, whose females do not produce that pheromone. We identify a gene that has evolved to reshape the neural circuit that processes this pheromone and change the male's response from attraction to repulsion. We assess how differential regulation of this gene in specific neurons changes the anatomical and physiological properties of the circuit between the species to produce divergent behaviors. This provides a unique mechanistic understanding of the genetics underlying neural circuit evolution.

1803F **Metabolic Dysfunction in Frontotemporal Dementia** Angelina Freeman[1], Ana Penagaricano-Abreu[1], Valentina Torres-Cerda[1], Marla Tipping[2] [1]Biology, Providence College, [2]Providence College

Frontotemporal dementia (FTD) is a neurodegenerative disease characterized by changes in behavior, a decline in executive function, and a loss of language abilities. With increased awareness that metabolic changes often present in disease, there has been a shift toward investigating the metabolic etiology of diseases, such as cancer. However, this shift is not as prevalent in the study of neurodegenerative disease. Using a *Drosophila melanogaster* model, we are studying the connection of neurodegeneration with the dysregulation of lipid metabolism associated with FTD. We utilized staining to visualize lipid stores, metabolic assays to quantify triglyceride levels, behavior analysis to monitor activity levels on a light-dark cycle, and quantitative PCR and western blots to measure other metabolic factors. The goal of this project is to evaluate the dysregulation of lipid metabolism in FTD and determine targets for potential treatments.

1804F **Hemocyte dependent regulation of alcohol induced responses as a function of temperature** Madhavi Kuchibhotla[1], Airined Montes[1], Josue A Rodriguez[1], Carolina I Maldonado[1], Raoul A Lebron Rivera[1], Dr.Jose L Agosto[1], Dr. Alfredo Ghezzi[1], Dr. Tugrul Giray[1], Dr. Maria E Perez Hernandez[2] [1]Biology, University of Puerto Rico, Rio Piedras Campus, [2]Mathematics, University of Puerto Rico, Rio Piedras Campus

Alcoholism is the third leading cause of deaths. One of the key components in the development of alcoholism is the gradual increase in resistance to alcohol over repeated exposures. This adaptation is also known as tolerance, which is thought to involve neural adaptations and plasticity within the brain leading to alcohol use disorders (AUD). Recently, the Neuroimmune system has emerged as an important player in the development of AUD. Well Established studies on polymorphisms in macrophages and immune genes were linked to higher risk of alcoholism,supporting the role of immunity and neuroimmune signaling in alcohol addiction. Drosophila studies have identified the RNA binding protein pumilio (pum) as a regulator of ethanol tolerance. Pum has also been linked to innate immunity and alcohol tolerance. The effects of temperature on sensitivity and tolerance have not been previously studied. We are inclined to hypothesize the tolerance might increase with higher interaction of dose just as doses alone to tolerance. Therefore, we were motivated to fill this knowledge.Thus, the overall goal of this study is to evaluate the role of pumilio within hemocytes in alcohol induced behaviors as the function of temperature. One of our focuses is to understand how pumilio can alter alcohol responses with temperature. For which, we employed GAL4/UAS binary system to generate targeted expression of GFP and/or pumRNAi transgenes precisely in hemocyte-specific driver line (hml-GAL4). We performed a 2-day ethanol assay paradigm on F1 age matched female Drosophila

melanogaster flies and sensitivity and tolerances were calculated using recorded sedation scores. All flies were subjected to 50% ethanol at 3 different temperatures (18°C, 22°C and 27°C) at 3 different instances. The focus of the study is to reveal the relation between ethanol responces and temperature. Our results indicate that, within the genotype, sensitivity to ethanol increases with temperature and pumilio knockdown increases sensitivity with temperature. Pumilio knockdown demonstrated an significant increase in tolerance index with temperature ($p<0.0002$) as opposed to its corresponding control flies, which were also statistically significant ($p<0.0001$). Overall, our data suggests that neuronal adaptation mechanisms underlying ethanol tolerance might be different from the perspective of dose only vs interaction of doses and environmental temperature. More specifically, at colder temperatures, one needs high doses of ethanol to be able to develop tolerance, suggesting the nervous system is less excitable and therefore needs more alcohol to be able to trigger plasticity mechanisms.In Contrast, high doses will not develop tolerance as much as at lower, suggesting high temperatures and high ethanol doses too much excitability is observed and plasticity mechanisms are blocked.

1805F **Contribution of Painless and TrpA1 to *Drosophila melanogaster* nociception** Jacob Jaszczak[1], Avi Simon[2], Luke Breuer[3], Nilson Palma[4], Susan Younger[2], Lily Jan[2], Yuh-Nung Jan[2] [1]New Mexico State University, [2]HHMI at the University of California, San Francisco, [3]Better Tools for Scientists, [4]City College of San Francisco

Nociception, the sensory mechanisms which respond to potentially damaging stimuli, depends on the coordination of multiple sensory channels. Understanding how multiple channels contribute to pain perception is critical for the management of chronic pain. *Drosophila melanogaster* larva exhibit a nociceptive escape behavior in response to a range of noxious temperatures (greater than 40°C) through the activation of two channels, Painless and TrpA1. These channels are both expressed in peripheral C4da neurons, which envelop the larval cuticle through their dendritic arbors. While both Painless and TrpA1 are thermally activated *in vitro*, different *painless* alleles have been reported to have distinct effects on larval nociceptive behavior, and we find that mutations of *painless* do not enhance the *TrpA1* phenotype. We compared the larval thermal nociceptive phenotypes of different *painless* alleles over a range of nociceptive temperatures. We find the alleles that have the strongest loss of nociceptive behavior also retain some RNA or protein products, whereas the *painless* alleles that do not retain detectible RNA or protein products do not lose their nociceptive phenotype. Ongoing work aims to create isoform specific and neomorphic *painless* mutants in order to test their contribution to the disruption of thermal nociceptive behavior and coordination with TrpA1 function.

1806F **Spatial transcriptomics reveals region-specific and cell-type-specific gene dysregulation in a *Mecp2* mouse model of Rett syndrome** Young Y Zhou[1], Julie Ruston[1], Bharti Kukreja[2], Nareh Tahmasian[2], Brian Kalish[2,3,4], Monica J Justice[1,3] [1]Genetics and Genome Biology, The Hospital for Sick Children, [2]Neuroscience and Mental Health, The Hospital for Sick Children, [3]Department of Molecular Genetics, University of Toronto, [4]Department of Paediatrics, University of Toronto

Rett syndrome (RTT) is a rare neurometabolic disorder primarily attributed to mutations in the X-linked methyl-CpG-binding protein 2 (*Mecp2*). In female RTT patients, a period of seemingly normal development spans from 6 to 18 months, followed by a gradual regression of speech and motor skills, accompanied by stereotypic hand movements, movement disorders, and sleep disturbances. While heterozygous (*Mecp2/+)* female mice are more clinically relevant, hemizygous male mice (*Mecp2/Y)* are the preferred model due to their penetrant phenotype. Notably, in male mice, the phenotype becomes evident at 4-5 weeks of age, although molecular perturbations manifest earlier. Our previous results from RNAseq and immunofluorescence experiments unveiled a critical developmental ‹switch› within the *Mecp2* brain, occurring between postnatal day 14 and postnatal day 21, leading to the onset of RTT symptoms. To illuminate the precise timing and cellular underpinnings of this ‹switch,› we harnessed MerFISH, a spatial transcriptomic technology with high spatial resolution and sensitivity. Employing this approach, we discerned distinct region-specific and cell-type-specific differentially expressed genes associated with cholesterol synthesis, signaling pathways, synaptic function, development, circadian rhythms, and various other vital processes. These dysregulated genes potentially hold developmental significance in the *Mecp2* brain, which may underlie the fundamental pathophysiology of RTT.

1807F **Genetic testing in children with developmental delay** Junghyeon Park[1], Ah Yeon Lee[1], Hye Jung Park[1], Myungshin Kim[2], Hoon Seok Kim[2], Seung Bin Lee[3], Joo Hyun Park[1] [1]Department of Rehabilitation Medicine, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, [2]Department of Clinical Laboratory Medicine, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, [3]Industry-Academic Cooperation Foundation, The Catholic University of Korea

Background

In recent years, molecular advancements greatly enhanced our capacity to identify genetic factors contributing to developmental delays in children, particularly in cases that were previously unexplained. This single-center study in South

Korea focused on reviewing cases of pediatric patients with developmental delays, with the aim of identifying the conducted genetic tests and assessing the results for children whose developmental delays had unclear origins.

Methods

We reviewed the records of pediatric patients who were genetically tested with developmental delays, hypotonia, or facial dysmorphism from 2009 to 2022. We enrolled 306 patients who underwent genetic testing, including karyotype analysis, chromosomal microarray analysis (CMA), targeted Next Generation Sequencing (NGS)-based gene panel (985 genes), Multiplex Ligation-dependent Probe Amplification (MLPA), and Fluorescence In Situ Hybridization (FISH).

Results

Among the 323 patients, 211 (65.3%) were male, and 112 (34.7%) were female. The average age at which genetic testing was conducted was 3.89 years old. Seventeen patients did not undergo the testing despite it being prescribed. The remaining 306 patients completed the tests as recommended. Sixteen patients carried karyotypic abnormalities (16/301). Abnormal CMA results were found in a total of 84 patients (32.7%, 84/257). Among these, 42 had microdeletions, 33 had microduplications, and 9 had both abnormalities. In the targeted NGS panel, mutations were detected in 50 out of 97 patients (51.5%). Among these, 4 were classified as pathogenic, 12 as likely pathogenic, and 34 as variants of uncertain significance (VUS). In MLPA, 2 out of 12 patients exhibited anomalies, while in FISH, anomalies were observed in 6 out of 7 patients. Parental testing was recommended for a total of 98 cases, and it was conducted in 77 cases.

Conclusion

Various genetic tests have demonstrated their utility in identifying the underlying causes of unexplained developmental delays. In children with developmental delay, if a genetic disorder is suspected based on the phenotype, more comprehensive testing such as WES (Whole Exome Sequencing) or WGS (Whole Genome Sequencing) may be necessary.

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1808F **Multi-omic interactions of multi-generational early life stress in developing zebrafish** Erik D Norloff[1], Yingning Sang[1], Oksana Kosyk[2], Darius Thompson[1], Anthony S Zannas[2,3,4], Christina L Graves[1,4] [1]Oral and Craniofacial Health Sciences, University of North Carolina at Chapel Hill, [2]Psychiatry, University of North Carolina at Chapel Hill, [3]Genetics, University of North Carolina at Chapel Hill, [4]Carolina Stress Initiative, University of North Carolina at Chapel Hill

Background: Chronic early life stress (ELS) is appreciated to be a powerful force in shaping biological outcomes later in life and is associated with the onset of gastrointestinal disease in humans. Further, recent longitudinal cohort studies demonstrate that multigenerational adversity impacts the gut microbiome composition in early childhood, highlighting the gut-brain axis as an important target of ELS. Building on our recently published work demonstrating that chronic ELS alters the neuroimmune profile and functioning of the developing zebrafish gut, our goal in this study was to establish a model of multigenerational ELS in zebrafish and determine cumulative stress impact on multivariate outcomes. **Methods and Results:** Wild-type AB zebrafish were exposed to chronic mild environmental stressors beginning at 6 dpf until 30 dpf according to our recently published stress paradigm for a total of four successive generations. We compared stressed and unstressed groups from either stressed or unstressed ancestors and found that chronic ELS was associated with reduced reproductive fitness, smaller size, and increased whole body cortisol with effects largely dependent on within-generation stress exposure. RNA-sequencing of gut tissue revealed ELS-associated differential expression of more than 800 genes in founder generations, some of which have been identified as susceptibility loci in human inflammatory bowel disease. **Conclusions:** Our data demonstrate that zebrafish are a powerful model for exploring neuroimmune interactions at mucosal surfaces across generations.

1809F **Motor Circuit Critical Period Closure in a Highly Regenerative Vertebrate** Haley Jetter, Jacob P Brandt, Sarah D Ackerman Pathology and Immunology, Washington University School of Medicine

An essential feature of nervous system development is the remodeling of circuits during critical periods of plasticity, brief developmental windows when the activity of neurons can modify circuit architecture and function to produce sustained changes in animal behavior. In *Drosophila,* astrocytes have been identified as key regulators of critical period closure in the invertebrate motor circuit, where astrocytes signal to motor neurons via a Neuroligin-Neurexin signaling axis to stabilize the developing motor circuit. Here we seek to characterize motor plasticity in zebrafish, an animal capable of complete motor circuit regeneration following injury. Whether a critical period exists, and what roles astrocytes have in regulating motor plasticity, remains to be investigated in a highly regenerative vertebrate model like zebrafish. In both *Drosophila* and mouse,

astrocyte-synapse contact is predictive of critical period closure. In zebrafish, astrocytes extend their initial processes at 2 days post fertilization (dpf) and show a mature morphology by 6 dpf. Thus, we utilize *in vivo* timelapse imaging and optogenetics in zebrafish between 2- and 6dpf to characterize activity-dependent motor neuron dendrite dynamicity. We hypothesize that it is during this developmental window, plasticity will be enriched, and that beyond 6 dpf, the presence of mature astrocytes will limit motor plasticity. We will then use CRISPR/Cas9 to knock out Neuroligins in astrocytes and measure the consequence to motor plasticity. We expect that loss of this astrocyte anti-plasticity cue will enhance motor plasticity, irrespective of whether plasticity is developmentally restricted. Together, we work toward identifying the roles of astrocytes during critical period closure in a highly regenerative vertebrate system.

1810F **The transcription factors *prdm16* and *prdm3* antagonistically regulate acoustic startle response thresholds** Zackary Q Marshall[1], Nicholas Santistevan[1], Susannah Schloss[1], Lomeli Shull[2], Kristin Artinger[3], Jessica C Nelson[1] [1]Cell and Developmental Biology, University of Colorado Denver, [2]Craniofacial Biology, University of Colorado Denver, [3]Diagnostic and Biological Sciences, University of Minnesota

An organism's survival depends on its ability to appropriately filter relevant sensory stimuli from the environment. Differences in sensory filtering exist in many neurological disorders such as migraine, schizophrenia, and ADHD. More specifically, migraine sufferers exhibit altered brain activity and decreased thresholds for visual, thermal, and acoustic stimuli even during interictal periods that intervene migraine attacks. Despite migraine being a leading cause of disability among women, the underlying genetic mechanisms remain elusive. From a pilot screen of putative migraine-associated genes, we uncovered a novel function for the transcription factor *prdm16* in regulating acoustic startle sensitivity. Previous work demonstrated that *prdm16* and *prdm3* orchestrate craniofacial development despite *prdm16* promoting Wnt/ß-catenin signaling and *prdm3* antagonizing Wnt/ß-catenin signaling. In a similar manner, we show *prdm16* homozygous mutants and crispants exhibit heightened acoustic startle sensitivity while *prdm3* crispants display severely reduced acoustic startle sensitivity. Interestingly, *prdm16* homozygous mutants injected with *prdm3* guides have startle thresholds that resemble their wild-type, control guide-injected siblings, indicating that an appropriate balance between *prdm16* and *prdm3* activity is required to tune sensory thresholds. Finally, to investigate which brain regions *prdm16* and *prdm3* modulate, we performed an unbiased whole brain activity mapping experiment, and found that whole brain activity of *prdm16* mutant larvae display hyperactivity in the habenula and the medial rhombencephalon as compared to their siblings. In contrast, we observe wide-spread hypoactivity in *prdm3* crispants, including within the habenula and the medial rhombencephalon. This work reveals a role for genes that balance Wnt/ß-catenin signaling in establishing appropriate sensory thresholds and provides proof of concept for a more comprehensive screen of migraine-associated genes, investigating how they might impact sensory thresholds *in vivo.*

1811S **Understanding the Impact of Superoxide Dismutase on Extracellular Vesicle Biogenesis and Cargo Sorting** Nahin Siara Prova[1], Jessica Tanis[2] [1]Biological Sciences, University of Delaware, [2]Biological Sciences, University Delaware

Extracellular vesicles (EVs), released by nearly all cell types, mediate intercellular transport of protein, RNA, metabolite, and lipid cargo that cannot readily cross the plasma membrane to recipient cells. Dictated by the site of origin, the cargoes packaged into EVs regulate normal physiological processes and propagation of pathological conditions. The cellular factors that modulate EV biogenesis and the enrichment of cargoes into distinct EV subpopulations remain unclear. EVs are released from cilia of *C. elegans* male sensory neurons, then are taken up by surrounding glia or released into the environment. The ion channel CLHM-1 is packaged into EVs shed from the periciliary membrane compartment of the ciliary base, while the TRP polycystin channel PKD-2 is found in EVs that bud from the cilium distal tip. To define factors that impact EV cargo loading and biogenesis, we image and quantitate the number of EVs released from animals that express tdTomato-tagged CLHM-1 and GFP-tagged PKD-2 at single copy. Accumulation of reactive oxygen species (ROS) is a byproduct of both physiological and pathophysiological processes in eukaryotic cells. If not neutralized, aging or pathogen-induced ROS production could potentially disrupt EV biogenesis and cargo sorting. Superoxide dismutase (SOD-1) is an antioxidant enzyme that acts as a ROS scavenger to help reduce oxidative stress. I found that loss of *sod-1* increases shedding of CLHM-1, but not PKD-2, EVs. CLHM-1 abundance in the ciliary base, but not cilium proper, was reduced in *sod-1* mutants. Thus, loss of *sod-1* differentially regulates release of different EV subpopulations, specifically increasing EV shedding from the ciliary base, which could impact neuron-glia communication. Achieving an understanding of how ROS impact EV biogenesis and cargo sorting is important because oxidative stress is an underlying factor for numerous pathological conditions which are impacted by EV release.

1812S **EGL-4 Protein Kinase G modulates the Hypoxia Stress Response and GLR-1 glutamate receptor synaptic trafficking** Tatiana Popovitchenko[1], Eunchan Park[2], Yeshaswi Pulijala[3], Christopher G Rongo[2] [1]Genetics, Rutgers University, [2]Department of Genetics, Rutgers University, [3]Rutgers University

Hypoxia (oxygen deprivation) plays a central role in diverse human diseases, including ischemic stroke, myocardial infarction, and cancer. Metazoans respond to hypoxia using the conserved hypoxia stress response pathway, which regulates

gene expression and metabolism to allow for survival in low oxygen conditions. For the model organism *Caenorhabditis elegans*, exposure to hypoxia tends to be environmental (e.g., hypoxic niches in the soil). Hypoxia results in changes in *C. elegans* metabolism and behavior. Here, we describe a novel role for the Protein Kinase G (PKG) ortholog EGL-4 in the hypoxia response. EGL-4/PKG has a well-established role in sensory integration and adaptation, particularly regarding the sensation of food and control of foraging behavior. Using genomic editing to introduce fluorescent tags into the endogenous *egl-4* locus, we confirmed the diverse neuronal profile of EGL-4 expression. EGL-4 is expressed in the command interneurons, which regulate direction of locomotion and roaming versus dwelling locomotory behavior. A key mediator of command interneuron function is the AMPA-type glutamate receptor GLR-1, which is localized to synapses along the ventral cord of the command interneurons. Our lab previously showed that hypoxia exposure or *egl-9* loss of function mutations block the endosomal recycling of GLR-1 receptors to synapses, resulting in reduced GLR-1 synaptic localization and GLR-1-mediated reversals in locomotory behavior. We observed similar defects in GLR-1 synaptic localization and GLR-1-mediated behavior in *egl-4* mutants. Using an auxin inducible degron sequence, we have modified the endogenous *egl-4* locus to respond to TIR1/auxin-mediated protein turnover, which we are using to examine the site and timing of action of this kinase in the command interneurons. Taken together, our results suggest that EGL-4/PKG signaling modulates the hypoxia stress response by regulating neurotransmitter receptor synaptic trafficking, which increases our understanding of how the hypoxia response pathway is integrated with signaling systems that respond to other environmental cues.

1813S **The neuropeptide nlp-50 regulates stress-induced sleep in C. elegans** Caroline Curtin Saint Joseph›s University

Caroline Curtin (Saint Joseph's University)
Advisor: Matthew Nelson (Saint Joseph's University)

Sleep is evolutionarily ancient, conserved, and likely occurs in all animals with nervous systems. Essential functions have preserved it throughout evolution despite its disadvantages, most notably it renders animals vulnerable to threats. Survival is predicated on the ability to regulate transitions between sleep and sensory readiness; these mechanisms are not well understood. The stress response of the genetically-tractable nematode Caenorhabditis elegans consists of threat avoidance, followed by stress-induced sleep, thus models this broader question. The paired ADL sensory neurons regulate avoidance behaviors and sleep and we have shown that the G-protein coupled receptor npr-38 functions in the ADL neurons to regulate these opposing behaviors. npr-38 mutants initiate sleep later than wild-type controls and display less total sleep; both phenotypes are restored when npr-38 is expressed specifically in the ADL neurons. nlp-50 neuropeptides are expressed in the ADL neurons, and are required for sleep, however when nlp-50 was rescued in the ADL neurons, the total amount of sleep was even further reduced. This suggests that nlp-50 promotes arousal and possible avoidance from the ADL neurons. Currently, my project is focused on determining the site of action for nlp-50 during sleep by measuring sleep in animals in which nlp-50 is restored in a cell-specific manner.

1814S **Investigating a simple neural circuit regulating stress responses in C. elegans** Mary Frattara[1], Matthew Nelson[2] [1]Biology, Mary Frattara, [2]Biology, Saint Joseph's University

C. elegans exhibit a two-fold stress response featuring threat avoidance followed by a state of stress-induced sleep. These represent opposing yet essential behaviors; how animals coordinate them is unclear. Avoidance requires sensory neurons like the ADL and ASH, and a hub interneuron called RMG. Stress-induced sleep requires the ALA and RIS interneurons; the RMG may also regulate arousal from sleep. Our lab has identified a G-protein coupled receptor, npr-38, that is expressed in each of these cells and is required for avoidance, sleep, and arousal. We propose that a circuit composed of 6 cells (ADL, ASH, ALA, RIS, AIB, and RMG) coordinates the stress response and the transitions between opposing behavioral states. As a first step of testing this hypothesis, we expressed the histamine-gated chloride channel HisCl1 in the ADL and RMG. We are currently measuring each aspect of the stress response following chemogenetic silencing of the ADL, the RMG, and both the ADL and RMG. This research will set the groundwork for future studies to delve into other relevant neurons in the circuit.

1815S **Small size correlates with reduced running in response to alternating current in *C. elegans*** Annalise I Totten[1], Victoria Lam[1], Shane Calle[1], Zoe Midthun[1], Ling Fei Tee[2], Gabrielle Martin[1], Kotarou Kimura[2], Jared Young[1] [1]Mills College at Northeastern University, [2]Nagoya City University

In response to alternating current of 30V applied to the agar substrate, C. elegans increases movement speed (Tee et al., Genetics, 2023). This response could be a manifestation of a fear emotion in the worms, causing them to panic and attempt to escape. Previously, sma-1 had been identified as a likely contributor to this behavior. Three alleles of sma-1(e934, e30, and ru18) were tested for electricity response and all were found to be defective. On average, these sma-1 worms showed less increase in their movement speed compared to wild-type worms, and were less likely to leave a food lawn when exposed to electricity. We have tested hypotheses regarding the specific biological contribution of sma-1 to the electricity response,

including testing of other C. elegans mutants that share phenotypic similarities to sma-1 mutants. These phenotypes include small size and defective excretory canals. The effect of size on the electricity response was tested using different ages of wildtype N2 strain and mutants of different sizes, with smaller size correlating with decreased electricity response.

Tee LF, Young JJ, Maruyama K, Kimura S, Suzuki R, Endo Y, Kimura KD. Electric shock causes a fleeing-like persistent behavioral response in the nematode Caenorhabditis elegans. Genetics. 2023 Oct 4;225(2):iyad148. doi: 10.1093/genetics/iyad148.

1816S **The role of GPCRs in regulating sleep through Notch signaling in C.elegans** Manuel F Lamela[1], Adam Michael Friedberg[2] [1]Neuroscience, Brown University, [2]Brown University

Sleep is an essential behavior observed across species and is characterized by behavioral quiescence, including reduced sensory responsiveness and decreased activity. In C. elegans, Notch signaling regulates developmentally timed sleep (DTS) which occurs in the transition between developmental stages during periods known as lethargus. A double knock-out of the two Notch co-ligands (osm-7 and osm-11) leads to a decrease in DTS, whereas overexpression of OSM-11 in adult C. elegans results in anachronistic sleep, a temporary, reversible behavioral quiescence similar to lethargus sleep (Singh 2011). With this knowledge, a forward genetic screen was undertaken to identify genes whose perturbation disrupts anachronistic sleep in C. elegans, and goa-1, a gene that encodes a G-alpha(o), was identified (Huang 2017). However, the GPCR receptors that may interact with GNAO1 to regulate sleep are still unknown. Notably, the orthologous human gene GNAO1 was associated with insomnia in a large-scale, human genome-wide association study, suggesting GNAO1 may be important in sleep across species (Lee 2019). To study this receptor in C. elegans, I (1) optimized the sleep assay for assaying anachronistic sleep presented in Huang et al. 2017, (2) CRISPR individuals with the transgene that I wanted to test (3) tested this mutant strain for sleep defects. The results will provide insight into interactions between GPCR and Notch signaling in regulating sleep, demonstrate conservation of GPCRs in C. elegans, and may provide mechanistic insights into the importance of GPCR signaling in sleep and insomnia.

Huang H, Zhu CT, Skuja LL, Hayden DJ, Hart AC. Genome-Wide Screen for Genes Involved in Caenorhabditis elegans Developmentally Timed Sleep. G3 Genes|Genomes|Genetics. 2017;7(9):2907-2917. doi:10.1534/g3.117.300071

Koelle MR, Horvitz HR. EGL-10 Regulates G Protein Signaling in the C. elegans Nervous System and Shares a Conserved Domain with Many Mammalian Proteins. Cell. 1996;84(1):115-125. doi:10.1016/S0092-8674(00)80998-8

Singh K, Chao MY, Somers GA, et al. C. elegans Notch Signaling Regulates Adult Chemosensory Response and Larval Molting Quiescence. Current Biology. 2011;21(10):825-834. doi:10.1016/j.cub.2011.04.010

1817S **A genetic screen for modifiers of stress-induced sleep in *C. elegans* using a worm picking robot** Zihao John Li[1], Kerry FY Lecure[2], David M Raizen[2], Chris Fang-Yen[3] [1]Bioengineering, University of Pennsylvania, [2]Neurology, University of Pennsylvania, [3]Biomedical Engineering, Ohio State University

Animals sleep more when they are sick. In *C. elegans*, stress-induced sleep (SIS) is regulated by epidermal growth factor (EGF) activation of the ALA and RIS neurons, which then release neuropeptides to trigger sleep. However, the mechanisms by which EGF is regulated and components acting downstream of ALA/RIS signaling are poorly understood. To gain insight into the mechanism of SIS, we conducted a genetic screen for modifiers of UV-induced sleep. We used the Million Mutation Project (MMP), a library of 2007 mutagenized and sequenced strains. The screen requires manipulating and phenotyping a large number of clones, making the experiments labor intensive. To increase throughput, we used our recently developed worm-picking robot to maintain the strain library, prepare sleep assays in the multi-well WorMotel platform, and perform other manipulations. Using the resulting sleep data from MMP strains, we developed statistical methods for determining the associations between the genes and sleep phenotypes. Our analysis unveiled a set of novel candidate genes associated with SIS in *C. elegans*. Further analysis of these genes holds promise to deepen our understanding of sickness sleep. In addition, our screening pipeline serves as a model for accelerating other genetic screens through robotic automation.

1818S **Response to two attractive odorants relies on distinct regulators of RNA silencing** Samiha Tasnim, Antony M Jose University of Maryland

Behavioral adaptation to dynamic environments is a hallmark of all animals. Although such adaptation has been proposed to require gene regulation, the underlying mechanisms are not well understood. The nematode *C. elegans* is a useful model for analyzing adaptation upon prolonged exposure to attractive odorants. It uses the AWC pair of amphid sheath neurons to respond to the attractive odorants butanone and benzaldehyde. It can adapt to each odorant independently such that prolonged exposure to either odorant leads to loss of attraction to that odorant. Inspired by prior reports on the role for RNA silencing in the response to butanone, we examined both sensation upon acute exposure and adaptation upon prolonged

exposure to butanone or benzaldehyde by animals lacking different regulators of RNA silencing. We generated null mutants of each regulator using Cas9-mediated genome editing and tested their behavior using a choice assay that we adapted to ensure a large effect size. Surprisingly, two proteins with roles in the germline were required for the response to both odorants. The nuclear Argonaute HRDE-1 was required for sensing both odorants and the Maelstrom-domain protein RDE-10 was required for adapting to both odorants. This requirement either reflects additional roles for these proteins in neurons or altered neuronal development resulting from loss of RNA regulation in the parental germline. Furthermore, the adaptation to benzaldehyde but not to butanone required the primary Argonaute RDE-1 and the somatic nuclear Argonaute NRDE-3. This selectivity suggests that the changes in RNA regulation required for adapting to butanone could be different from those required for adapting to benzaldehyde. Together, our results reveal that the response to two attractive odorants sensed by AWC neurons share RNA regulators with known roles in the germline and additional distinct RNA regulators.

1819S **Neurofibromin deficiency alters the initiation and perseveration of temporally-sequenced behaviors** Genesis Omana Suarez[1], Divya S Kumar[2], Hannah Brunner[1], Jalen Emel[1], Valentina Botero[1], Connor Broyles[1], Aaron Stahl[1], Salil S Bidaye[2], Seth M Tomchik[3] [1]University of Iowa, [2]Max Planck Florida Institute for Neuroscience, [3]Neuroscience and Pharmacology, University of Iowa

Neurofibromatosis type 1 (NF1) is a genetic disorder that drives tumor formation and affects brain function in some patients. The disorder produces a range of symptoms, including increasing susceptibility to cognitive and behavioral symptoms such as autism spectrum disorder and attention-deficit/hyperactivity disorder. This suggests that NF1 affects neuronal function, leading to changes in behavior. NF1 is caused by loss of function mutations in the *NF1* gene and its protein product, neurofibromin. To dissect the mechanisms of how NF1 affects neuronal function, we have examined the effect of neurofibromin deficiency (mutants/RNAi) on motor function in *Drosophila*. Loss neurofibromin generates behavioral phenotypes, including increasing the frequency of spontaneous grooming. Here we report that neurofibromin deficiency alters the patterning and prioritization of temporally-sequenced motor behaviors. Spontaneous grooming was increased in a nonlinear spatial and temporal pattern, with grooming of certain body parts selectively elevated in a time-varying manner. This was state-dependent, as food deprivation resulted in a genotype-dependent behavioral switch from a grooming- to foraging-dominant behavioral mode over time. Loss of Nf1 in discrete sensory circuit and/or command neurons did not alter grooming frequency, though in some cases, it biased spontaneous grooming toward a specific body part. Walking patterns were altered - flies lacking Nf1 walked faster, but without significant alteration in leg kinematics or interleg coordination. Overall, these data suggest that neurofibromin deficiency modulates neuronal network activity nonlinearly, altering the selection of temporally-sequenced behaviors.

1820S **Long non-coding RNA Statera regulates synaptic plasticity at the *Drosophila* neuromuscular junction** Shuhao Wang, Gimena Alegre, Travis Thomson UMass Chan Medical School

It is widely accepted that synaptic plasticity is the foundation of learning and memory and perturbation of plasticity is implicated in many neurological and psychiatric diseases. Our lab previously described a protein and RNA trafficking pathway ViSyToR (Viral Synaptic Transfer of RNA) required for synaptic plasticity at the Drosophila neuromuscular junction (NMJ). Central to this pathway are two capsid genes, dArc1, a Drosophila homolog of Arc (Activity-Regulated Cytoskeleton-Associated protein) which is a positive regulator of plasticity, and Copia, a retrotransposon which is a potent negative regulator of plasticity. Using RNA immunoprecipitation, we found that the long non-coding RNA Statera is bound by dArc1 and Copia proteins. By analyzing existing single-cell RNA-Seq datasets, we found that Statera is universally expressed in Drosophila and induced during aging. RNA FISH data shows that Statera is localized to the CNS, as well as the nucleolus of muscle cells. Neuron-specific Statera knockdown by RNAi and whole-body Statera knockout by CRISPR both lead to a decrease of bouton numbers at the NMJ, suggesting a role of Statera in synaptic plasticity. Furthermore, RNA-Seq and dPCR data show that Statera knockout leads to an increase of a group of transposable elements including Copia. Taken together, our results point to an unexpected role of a long non-coding RNA in synaptic plasticity, possibly by regulating the levels of transposable elements.

1821S **Recurrent spontaneous seizures caused by glial-*Dube3a* overexpression can be suppressed through modulation of 5-HT signaling** Saul Landaverde[1], Reid Schuback[1], Megan Sleep[1], Selene Tan[1], Andrew Lacoste[1], Lawrence T. Reiter[2,3], Atulya Iyengar[1] [1]Department of Biological Sciences, The University of Alabama, [2]Department of Neurology, The University of Tennessee Health Science Center, [3]Department of Anatomy and Neurobiology, The University of Tennessee Health Science Center

The E3 ubiquitin ligase *UBE3A* catalyzes the transfer of ubiquitin to substrate proteins marking them for proteolysis. Over-expression of *UBE3A* is thought to contribute to Duplication 15q syndrome since *UBE3A* is located within the 15q11.2-q13.1 duplication. Dup15q patients often display autism, muscle hypotonia, and epilepsy. In vertebrate models, overexpressing *UBE3A* in neurons recapitulates several aspects of Dup15q syndrome, but not epilepsy phenotypes. Previous work in Drosophila indicates that overexpression of *Dube3a* (homolog of *UBE3A*) in glia leads to a “bang-sensitive”

hyperexcitable phenotype. Here, we used the Gal4-UAS system to compare overexpression of *Dube3a* in glial and neuronal cells (using the *repo*-Gal4 and *nsyb*-Gal4 drivers respectively) to determine if either manipulation led to spontaneous seizure phenotypes. In an intact tethered fly preparation, we recorded action potentials from the indirect flight muscles (dorsal longitudinal muscles, DLMs). We found recurrent, spontaneous spike discharge in *repo* > *Dube3a* flies ($\bar{x}$ = 2.19 Hz; 0.387 burst freq. min^{-1}) but not *repo* > w^{1118} control flies ($\bar{x}$ = 0.11 Hz; 0 burst freq. min^{-1}). These bursts originated centrally and were correlated between the left and right sides. Furthermore, blocking central excitatory neurotransmission (via the nAChR antagonist Mecamylamine) stopped bursting activity. In contrast, we did not observe spontaneous spike discharges in *nsyb* > *Dube3a* flies. These studies confirm previous work suggesting that glial rather than neuronal overexpression of *Dube3a* is the main contributor to spontaneous seizure activity. The lack of effective treatment options for Dup15q-associated epilepsy presents a hurdle in seizure management. Previous work has identified, Vortioxetine (a 5-HT1A agonist) and Ketanserin (5-HT2A receptor antagonist) as potential treatment options for Dup15q-associated epilepsy. We reared *repo* > *Dub3a* and *repo* > w^{1118} flies with these pharmacological agents (40nM and 400nM) to evaluate their efficacy in treating the seizure phenotype. We found both pharmacological agents were successful in eliminating spontaneous spike discharges in *repo* > *Dube3a* flies. Our findings highlight the potential for glial pathophysiology in underpinning seizures associated Dup15q syndrome.

1822S **Distinct growth property of *Drosophila* primary embryonic neurons derived from hyperexcitable K_v and Na_v channel mutants** Yeo Rang Lee, Jessie Shirley, Cameron Mahanke, Atulya Iyengar Department of Biological Sciences, The University of Alabama

Ion channels play essential roles in action potential propagation and cellular homeostasis in neurons. In *Drosophila*, mutations in genes that encode voltage-gated ion channels can lead to excessive and abnormal neuronal activity. Both loss-of-function mutations in voltage-gated potassium (K_v) channel genes *ether-a-go-go* (*eag*) and *Shaker* (*Sh*) as well as recently-identified gain-of-function mutations in the sole voltage-gated sodium (Na_v) channel gene *paralytic* (*para*) show hyperexcitable phenotypes such as vigorous leg shaking under ether anesthesia and impaired flight. Hyperexcitable K_v channel mutations also affect the growth and maturation of neurons, with primary cultured *eag Sh* neurons displaying elevated branching and growth cone complexity. Although effect of K_v channel mutations on neuronal growth are documented, it is unclear whether hyperexcitable Na_v channel mutants also display similar neuronal development features or if new neuronal growth phenotypes emerge. We undertook longitudinal studies of neuronal morphology comparing embryonic primary neuronal cultures of hyperexcitable Na_v and K_v channel mutants. Consistent with previous reports, we found *eag Sh* K_v double mutant neurons showed enhanced neurite terminal complexity and growth cone size. These phenotypes were exaggerated upon rearing at high temperature (30℃). In contrast, hyperexcitable *para* alleles displayed heterogenous collection of phenotypes. We found the hyperexcitable Na_v mutant, $para^{bss1}$, showed increased neurite terminal complexity while $para^{GEFS+}$ and $para^{Swd}$ did not show this phenotype. Unlike *eag Sh*, at room temperature, none of the Na_v mutants showed changes in growth cone size. However, at high temperature, both $para^{GEFS+}$ and $para^{Swd}$ mutants showed enlarged growth cones while $para^{bss1}$ did not. Together, our results highlight the complex relationship between excitability properties and neuronal development.

1823S **Investigating variability in learning and memory phenotypes using the *Drosophila* Synthetic Population Resource** Victoria Hamlin[1], Elizabeth King[2] [1]Biological Sciences, University of Missouri, [2]University of Missouri

Learning and memory are complex cognitive traits with function impacted by many factors including genomic makeup, environmental factors, and gene-environment interactions. These traits are vital for an organism's survival in an ever-changing environment by allowing for behavioral modifications in response to a stimulus. Within a given population, we see a wide range between individuals' ability to perform different cognitive tasks, however, the mechanisms underlying this variability are still largely unknown. Our goal is to identify the genomic regions contributing to cognitive performance and the effects of genomic variation on synaptic morphology and plasticity in the neural networks involved.

In our work we use a multifaceted approach to identify the important genomic regions contributing to the variability in cognitive traits. Our first approach uses Recombinant Inbred Lines (RILs) from the *Drosophila* Synthetic Population Resource (DSPR), a multiparent population consisting of about 1800 RILs, which provides us with the ability to perform high resolution genome wide scans. Through behavioral phenotyping of the RILs, we can identify genomic regions which significantly contribute to both the mean of a phenotype (mQTL), the residual variance of a phenotype (vQTL), or a combination of the two (mvQTL). Second, we took an evolve and resequence approach to identify changes in allele frequencies in our selected and control population lines. Both approaches used an appetitive olfactory conditioning Y-maze assay in which flies were trained to associate a specific odor with a sugar reward after dietary restriction. So far, we have identified several suggestive QTLs to investigate further to identify potential candidate genes. By linking genotype to phenotype, we will further our insight into the mechanisms of variability within the nervous system leading to a greater understanding of formation, function, and dysfunction of circuits involved in cognitive tasks.

1824S **The conserved microRNAs *miR-34* and *miR-277* regulate proportional growth of the neuromuscular junction in *Drosophila melanogaster* larvae** Mala Misra, Lauren T Maynor, Jackson J Sopa, Hillary P Hwang, Zeeta A Abubakar Biology, Washington College

As organisms grow, neurons maintain innervation of target tissues through proportional growth and elaboration. This scaling growth is regulated by a combination of global hormone signaling and cell-autonomous gene regulation, but our understanding of the interplay between these mechanisms and the genes involved is incomplete. In *Drosophila melanogaster,* the neighboring microRNAs *miR-34* and *miR-277* are part of a negative signaling loop with ecdysone, a circulating hormone linked to growth arrest during the larval-to-pupal transition. We examined the expression and function of these two microRNAs in the regulation of scaling growth of the neuromuscular junction (NMJ) during larval development. Quantitative PCR analysis in whole larvae and in isolated larval central nervous system tissue suggested that expression of *miR-34*, *miR-277*, and primary isoforms containing these microRNAs is higher during growth phases of larval development and drops off during late third instar, when larval growth is suspended at the onset of pupariation. Suppression of *miR-34* and *miR-277* function in motor neurons during larval development disrupts coordination of proportional NMJ growth by causing overgrowth of motor neuron axon terminals and by non-cell autonomously impairing target muscle growth. Our observations suggest that *miR-34* and *miR-277* act as signaling nodes in the regulation of proportional growth at the NMJ.

1825S **Matching transcription factor codes with morphological neuron types by lineage origin, neuron birth order, and spatial transcriptomics** Angelica Previero[1,2], Tzumin Lee[1,2] [1]Molecular, Cellular, and Developmental Biology, University of Michigan, [2]Life Science Institute, University of Michigan

In the brain, distinct neuron types express different combinatorial transcription factors (TF) codes, the identification of which is essential for elucidating the mechanisms of neuronal diversification and specification. While scRNA-seq permits comprehensive discovery of putative TF codes, finding their corresponding neuronal types in the complex brain remains tedious and often unachievable. In the Drosophila brain, which develops through stereotyped neuronal lineages, we conceived a strategy to match neurons' TF codes and morphologies systematically by placing gene expression patterns in the context of cell lineages. To realize this, we explore combining multiplexed smFISH with CLADES, a genetic cascade to label serially derived neurons with a predefined color sequence. I will present the application of this system to the mapping of the combinatorial TF codes for the 50 neuron types serially made by a common neural stem cell in the Drosophila central brain.

1826S **m6A-inhibitory RNA binding protein Nab2 Regulates Lipid Storage and Metabolic Pathways** Jordan Goldy, Anita Corbett, Kenneth H Moberg Emory University

The brain plays a critical role in regulating metabolism by sensing metabolic status and modulating release of metabolic hormones and regulators. This link is evident in clinical data as an elevated risk among individuals with intellectual disability (ID) for metabolic defects, including obesity, abnormally high triglycerides, and elevated blood sugar. One group of heritable forms of ID are caused by mutations in genes encoding RNA binding proteins (RBPs), which control RNA 5'-capping and polyadenylation, splicing, nuclear export, localization, and turnover. In some cases, RBPs with important and conserved roles in development of the central nervous system (CNS) also modulate metabolic status of distant organs, consistent with regulation of neuronal RNAs encoding secreted factors that modulate systemic metabolism. Notably, loss of function mutations in the RBP *ZC3H14* are linked to a form of non-syndromic, autosomal recessive human intellectual disability. Studies of the ZC3H14 ortholog Nab2 in *D. melanogaster* show that loss of Nab2 results in behavioral impairments, short-term memory deficits, reduced survival, altered brain morphology and increases in steady-state levels of various transcripts. Previous studies reveal a role for Nab2 in modulating the levels of N^6-methyladenosine(m^6A) deposition by Mettl3, the catalytic component of m^6A methyltransferase complex, on specific mRNAs. RNA-seq data indicate that two transcripts altered in *Nab2* null females are the insulin-like peptides *dilp2* and *dilp5*, which regulate systemic *Drosophila* metabolism and lipid storage. dIlp2 and dilp5 are synthesized in IPCs (insulin-producing cells), analogs of pancreatic beta cells in humans, and signal to cells in the fat body (FB) to promote lipid storage. Consistent with female-specific *dilp2/dilp5* data, preliminary analysis of *Nab2* null 3rd instar larvae reveals increased size of lipid droplets in female FBs, arguing that Nab2 is a female-specific inhibitor of lipid storage by virtue of its role in a brain-FB adipose storage circuit. Interestingly, a loss of Mettl3 in females results in an increase in *dilp2* transcript levels and increased average lipid droplet size comparable to the *Nab2* null females. Thus, we hypothesize that Nab2 regulates mRNA transcripts critical for proper metabolic function and lipid storage. We will discuss this evidence and present data on the mechanistic basis for this metabolic phenotype in *Nab2* null females.

1827S **Disruption in calcium conductance of cacophony channels alters VGCC abundance and retention at active zones** Chhavi Sood[1], Karen L Cunningham[2], Hannah B Hicks[2], J. Troy Littleton[1] [1]Picower Institute of Learning and Memory, Massachusetts Institute of Technology, [2]Massachusetts Institute of Technology

Active zones (AZ) are specialized presynaptic sites where voltage-gated calcium channels (VGCCs) mediate Ca^{2+} influx to trigger synaptic vesicle (SV) fusion and neurotransmitter release. A major determinant of SV release probability (P_r) and presynaptic output is the amount of presynaptic Ca^{2+} influx, which is correlated with the abundance of VGCCs at release sties. To better understand how VGCCs traffic, accumulate and recycle at AZs, we are using the *Drosophila* neuromuscular junction to characterize how the abundance of Cacophony (Cac), the sole AZ-localized VGCC in *Drosophila*, is set and regulated at individual release sites. We found that AZs accumulate Cac to a capped upper limit during AZ maturation, in contrast to the core AZ scaffold Bruchpilot (BRP), whose AZ abundance increases given extended developmental time. During larval development, Cac abundance varies across the AZ population and correlates with AZ size and synaptic output, and its delivery is rate-limited by the accessory subunit α2δ. Using photoconvertible mMaple-tagged endogenous Cac, we observed individual channels are largely confined to single AZs and their turnover is promoted by delivery of new Cac, which sets an upper limit on AZ Cac accumulation. In vitro studies in other models have suggested Ca^{2+} conductance properties may regulate VGCC trafficking and synaptic abundance, however, whether channel conductance also regulates in vivo trafficking and synaptic abundance of VGCCs is less clear. To assay if alterations in Ca^{2+} conductance provides additional layers of control for Cac abundance and turnover at AZs in vivo, we generated GFP and mMaple-tagged Cac conductance mutants (cac^{F} and cac^{NT27}) to compare their AZ dynamics to wildtype channels. We found that heterozygotes and homozygotes of *cac* conductance mutants showed increase in AZ abundance of both the mutant and wildtype channels. Furthermore, there was a concomitant increase in AZ area and abundance of BRP in the *cac* conductance mutants compared to wildtype. Ongoing studies will examine whether alterations in AZ abundance of Cac in the *cac* conductance mutants are due to disruptions in synaptic delivery, AZ retention and/or recycling of Cac during synaptic development and AZ maturation.

1828S **A conserved gene network enables decisions by regulating the distribution of an excitatory receptor** Kexin Zhang[1], Ornella P T Meko[2], Mandy Wong[2], Claudia M Mizutani[1], Rui Sousa-Neves[2] [1]Biology, Case Western Reserve University, [2]Genetics and Genome Sciences, Case Western Reserve University School of Medicine

Decisions plays a fundamental role for the survival of animals but little is known about the mechanisms that generate them. Previously we showed that the transcription factor DATILÓGRAFO (DATI) is required in cholinergic neurons in three brain regions of Drosophila females for the decision to accept courting males. Recently, we identified computationally the gene network regulated by DATI and validated several genes for their requirement in female acceptance using RNAi knockdown in cholinergic neurons. Among these genes, we find the receptor anchor CORACLE (CORA), the excitatory acetylcholine receptor nAChRa6, the translational repressor Bruno 3 (Bru3), and the regulator of transcription and RNA degradation, CG1677, here renamed *dati switch* (*ditch*). Here we tested if CORA and/or Bru3 are required for the localization of nAChRa6 in cholinergic neurons. We analyzed the expression patterns of nAChRa6 using 3D hotspots analyses and depth maps that provide 3D distribution statistics. Our data shows that CORA does not anchor nAChRa6 but it limits the access of this excitatory receptor to certain regions of dendritic neuropiles in the antennal lobe. Like CORA, Bru3 also limits the distribution of nAChRa6, suggesting that Bru3 and CORA are both part of a mechanism that prevents the expression of this excitatory receptor in specific regions of the antennal lobe and thus prevent the possibility of generating abnormal excitation. Finally, we show that DITCH is frequently found colocalized with Bru3 in the dendritic neuropile, suggesting an association between RNA repression and degradation. Together, these data identify conserved genes involved in decision making and show that they are part of a mechanisms that enables the allocation of receptors within neurons.

1829S **Defining Locomotor Deficits in KDM5 Loss of Function Mutants** Aubrey Siebels[1], Bethany Terry[2], Matanel Yheskel[2], Julie Secombe[2] [1]Neuroscience, Albert Einstein College of Medicine, [2]Genetics, Albert Einstein College of Medicine

The lysine demethylase 5 (*KDM5*) family of histone demethylases are crucial regulators of transcription. Loss of function mutations in the genes encoding the *KDM5* family of transcriptional regulators are associated with neurodevelopmental disorders. Among the observed missense mutations in the *KDM5* family, mutations in the *KDM5C* gene are among the best described. In the largest caregiver-reported questionnaire on the symptoms of patients with *KDM5C* variants, 87% and 81% of patients exhibit fine and gross motor delays, respectively. Drosophila offer an excellent opportunity to investigate the consequences and mechanisms of this phenomenon because they encode a single *KDM5* gene that likely reflects functions of all mammalian paralogs. It is unclear which components of locomotion planning and execution may be affected by *KDM5* mutations. To address this open question, we will begin by investigating the effects of cell-type specific KDM5 knock-down on Drosophila locomotion. Although the exact role of the *KDM5* family is not fully understood, it is increasingly evident that demethylase-independent functions of KDM5 may also play an important role in determining what genes are transcribed. We have developed a suite of *Drosophila* lines that carry missense mutations in different domains on the orthologous *KDM5* gene, some mutants have disrupted demethylase function, and some have intact demethylase domains. We will also examine variations in locomotion across these missense mutation strains to parse out the domains of *KDM5* that may be required for normal locomotor planning and execution. Establishing the nuances in locomotor functionality across the various missense mutation lines, serves as a crucial step into and we intend to expand into examining which domains and

functions of KDM5 may contribute to the motor delays seen in *KDM5C* variant patients.

[JS1]I would have this as a separate sentence, and perhaps start with the fact that the phenotypes associated with X-linked KDM5C are the best described (or something) … with 87/81% etc…

1830S **Effects of a Differential Overexpression of the Vesicular Acetylcholine Transporter on Synaptic Activity and Behavior in *Drosophila melanogaster*** Katarzyna D Rosikon, Benjamin D Church, Hakeem Lawal Delaware State University

Impairment in cholinergic neurotransmission is associated with normal and pathological aging, making cholinergic release a subject of sustained interest. However, the precise role of changes in central acetylcholine (ACh) release in mediating behaviors that range from locomotion to cognition has not been fully elucidated. The vesicular acetylcholine transporter (VAChT) is present in many species, including worms, flies, and humans, and is responsible for the packaging of ACh for exocytotic release. Although there is a plethora of knowledge about the molecular machinery that regulates ACh, the exact manner in which VAChT, an essential component of ACh regulation, alters ACh-linked neuronal function remains a subject of active investigation. Here, we use the overexpression of VAChT as a tool to increase the amount of ACh released into the synaptic cleft. And we are measuring the effect of that altered state on synaptic activity using two key behavioral circuits, locomotion and cognition. Previously, we showed that vast increases in VAChT expression cause severe behavioral deficits, including a sharp decline in lifespan. Our current study is focused on testing the hypothesis that more moderate increases in VAChT expression will not only lead to less severe effects but also beneficial ones. To test this idea, we used four VAChT overexpressing lines with varying levels of increased expression. We report the intriguing results that while strong increases in VAChT produced a corresponding decrease in lifespan, a less drastic overexpression of the protein led to a less steep decline in lifespan. Moreover, we show that in agreement with our previous published findings, our preliminary data show that VAChT overexpression caused an age-dependent decrease in locomotion ability in all lines tested. Further, immunohistochemical analysis showed that at least one VAChT overexpressor showed a strong increase in localization of the protein to punctate in the optic lobe, indicative of increased presence in synaptic vesicles. Taken together, these data indicate that morphological and behavioral effects of VAChT overexpression are driven by the levels of the protein's expression and inform further studies to be aimed at identifying precisely which dial in VAChT expression could lead to a beneficial effect on synaptic neurotransmission.

1831S **Effect of larval diet on adult feeding preference in *Drosophila Melanogaster*** Vaibhav D Menon[1], Christopher Creighton[1], Mayra Vazquez-Muñoz[2], Anupama A Dahanukar[1,3] [1]Neuroscience Graduate Program, University of California, Riverside, [2]Division of Biomedical Sciences, University of California, Riverside, [3]Department of Molecular, Cell and Systems Biology, University of California, Riverside

An animal's ability to taste is critical, underlying its selection for food sources that fulfill energetic and physiological needs, as well as rejection of potentially toxic substances. While the role of innate taste perception is well established, it is becoming increasingly clear that factors such as environment and internal state can influence taste-mediated behavioral outcomes. Yet, exposure to taste cues early in life remains a subject of limited analysis. We present a model for evaluating the role of larval taste input on adult feeding preference in *Drosophila melanogaster*. Our preliminary analysis identifies a pathway through which chemosensory stimuli elicit long-term modifications to feeding behaviors via exposure during a critical developmental period. We find that dietary exposure of larvae to known aversive taste compounds attenuates feeding avoidance of those same compounds in adult flies. The behavioral modification is specific in that it occurs with some compounds and not others, and selective in that its feeding preferences for other tastants, including closely related ones, are not affected. Notably, experience-dependent attenuation of feeding avoidance persists well into adulthood. Interestingly, proboscis extension responses show parallel changes, raising the possibility that developmental dietary experience with certain bitter tastants may impact the function of taste sensory function or circuits in a way that selectively reduces avoidance of those tastants. To understand developmental exposure-induced changes in bitter taste, we are taking a two-pronged approach by testing mutants of chemosensory genes that mediate bitter neuron activation and sweet neuron inhibition, and identifying chemosensory genes that are differentially expressed under naïve and tastant-exposed conditions. We find that bitter neuron activation and sweet neuron inhibition are both required to elicit experience-dependent changes to feeding preference. We are also investigating the role of long-term memory in shifting feeding behavior outcomes, using flies lacking neuronal activity in various mushroom body neuron populations. Together, our findings suggest a role for the gustatory system in eliciting persistent, robust changes in feeding behavior across metamorphosis and point towards a putative critical period for shaping taste preferences.

1832S **The DATI Neuroprocessor Enables Mating Decisions in *Drosophila* Female Through a Molecular and Cellular Mechanism that Eliminates Competing Behaviors** Mandy Wong[1], Ornella P T Meko[1], Kexin Zhang[2], Megan L Miller[1], Claudia M Mizutani[2], Rui Sousa-Neves[1] [1]Genetics and Genome Sciences, Case Western Reserve University School of Medicine, [2]Biology, Case Western Reserve University

Previously we identified DATILÓGRAFO (DATI) and showed that this transcription factor is required for decision of accepting male courtship in cholinergic neurons in three regions of *Drosophila* female brain. More recently, we screened the *Drosophila* and human genomes for genes containing DATI binding sites and found that 89% of the genes found in Drosophila are also present in the human genome. A systematic validation with RNAi interference in cholinergic neurons of 10% of these genes identified 12 novel genes involved in decision making. Remarkably, 41% of the genes validated either interact physically with the Amyloid Precursor Protein implicated in familial Alzheimer's Disease (AD), or are themselves candidates of other forms of AD, a disease characterized by memory loss, and decision-making deficits.

This validated blueprint equips neurons with the genes required to make decisions which we here refer to as the DATI neuroprocessor. This neuroprocessor contains two excitatory receptors (nAchRα6, nAchRα7), two inhibitory receptors (GluClα, Dop2R), a receptor anchor (CORA) and a low voltage calcium channel (Ca-α1T). Noteworthy, the loss of either the excitatory receptors that generate current or the inhibitory receptors that interrupt current results in rejection. It is then evident that these receptors excite and inhibit different parts of the neurons, working together to produce acceptance. Using 3D spatial analyses with spatial statistics, we show that these receptors have different localizations, and that CORA regulates the localization of nAchRα7, GluClα and Dop2R. In addition, we show that Ca-α1T is needed to secrete glutamate in the same cholinergic neurons that receive glutamate. This glutamate binds to GluClα, causing inhibition. Thus, the picture that emerges from these experiments is these are auto-inhibitory cholinergic neurons with an excitatory path that leads to acceptance and a separate path where signal must be blocked by an inhibition in order to prevent rejection from occurring. When uninterrupted, this separate pathway leads to signal loss through ShakB electrical synapses to neurons that process information about food. With this knowledge, we designed experiments that generate abnormal but predictable decisions and the results validated our arrangement of this neuroprocessor. Finally, we found that the Amyloid Precursor protein of *Drosophila*, Appl, not only interacts with proteins in this circuit but also is also required to make these decisions in this circuit. These findings reveal a conserved mechanism of decision making with direct relevance to our understanding of AD.

1833S **Dissecting the transcriptional networks driven by the Fra/DCC intracellular domain** Camila M Barrios-Camacho, Greg J Bashaw Neuroscience, University of Pennsylvania

Midline circuit assembly relies on the temporally- and spatially- regulated expression of axon guidance receptors. In *Drosophila*, the Frazzled receptor promotes midline crossing through netrin-dependent and netrin-independent mechanisms. In the former, commissural axons respond to Fra's chemoattractive ligand Netrin to project towards the midline. In the latter, γ-secretase cleaves the Fra intracellular domain (ICD), allowing it to translocate to the nucleus and induce the expression of *commisureless* (*comm*). *Comm* desensitizes crossing axons to the midline repellant Slit by inhibiting the trafficking of its Robo receptor to the plasma membrane. Once neurons have crossed, *comm* expression is down-regulated and sensitivity to repellants is re-established, thus facilitating commissural axon exit from the midline and simultaneously preventing their re-crossing. It is unknown if this *in vivo* transcriptional output of Fra is conserved in the human homolog, DCC, despite evidence that the ICD of Fra's vertebrate orthologs, Neogenin and DCC, also undergo γ-secretase-mediated cleavage and enter the nucleus to regulate transcription *in vitro.*

The P3 domain of the ICD functions as a transcriptional activation domain and in *Drosophila*, specific point mutations within this region render the receptor transcriptionally inactive, unable to induce *comm* expression. However, it is not known whether the role of these residues are conserved in the DCC-ICD. To test this, we generated various HA-tagged DCC-ICD mutant plasmids: 1) an ICD over expressor (ICD-OE) 2) a point mutant L1433A 3) a point mutant Q1426A 4) a dual point mutant L1433A/Q1426A, 5) a ΔP3 domain mutant and 6) an HA-tag negative control. With these plasmids, we generated stably transfected HEK293 cell lines and subjected these to bulk-RNA sequencing. Bioinformatic analyses show that transcriptomes of the ICD mutants are equivalent to that of the HA-tag negative control; however, over-expression of the wild-type ICD induces a distinct transcriptomic profile. We apply network topology approaches to this transcriptomic profile and identify mechanistically testable candidates. We are currently evaluating the expression patterns of these candidates and testing their roles in midline crossing.

1834S **Investigating the role of *NLGN3* in autism spectrum disorder and sleep disruptions** Rebekah Townsley[1,2], Jonathan Andrews[1,2], Sharayu Jangam[1,2], Michael Wangler[1,2] [1]Molecular & Human Genetics, Baylor College of Medicine, [2]Dan & Jan Neurological Research Institute, Texas Children's Hospital

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder that is highly comorbid with sleep disorders; however, the etiology of this co-occurrence is unknown. Recently, variants in the *Neuroligin* (*NLGN*) gene family have been identified in patients with both ASD and sleep disorders. NLGNs (NLGN1-5) are a family of postsynaptic cell adhesion molecules that interact with their presynaptic partner, Neurexins (NRXNs), to regulate synapse formation and maturation. They also indirectly recruit GABAergic and glutamatergic receptors to the postsynaptic membrane to establish the mature

neuron. *NLGN* members have unique expression patterns at either GABAergic or glutamatergic synapses; however, only *NLGN3* is expressed in both. We hypothesize that *NLGN3* variants impact GABA and glutamate activity resulting in ASD phenotypes and sleep disturbances. It is well known that the loss of *Nlg3*, the *Drosophila* homolog of human *NLGN3*, results in viable flies that have reduced locomotor activity; however, its effect on sleep and circadian rhythm is still unknown. We generated three *Nlg3* mutant alleles and found that two of the mutant lines exhibited a reduction in locomotor activity under a light/dark cycle. Furthermore, these same mutant flies also showed a decrease in the number of inactive periods and the periods of inactivity were longer compared to controls. Expression analysis also revealed that *Nlg3* is primarily colocalized to neurons and not to glia. Since human *NLGN3* variants have been reported to be associated with ASD and/or sleep disorders, we also functionally assessed these variants in the *Drosophila* model by generating transgenic flies expressing the human reference and variant cDNA. Leveraging the UAS-GAL4 system, we found that overexpressing either the human reference or variant cDNA did not cause any gross toxicity and the mendelian ratio was consistent for both males and females. Furthermore, the human reference and variant was unable to rescue the loss-of-function phenotypes. For future directions, we will be assessing the morphology and physiology of the neuromuscular junction. Our goal is to provide a functional link between ASD and sleep disturbances in order to identify potential therapeutic approaches to lessen the impact of sleep abnormalities in individuals with ASD.

1835S **Expression Analysis of cell-type-specific split-GAL4 lines in developing Drosophila visual system** Angelina Fordjour[1], David Chen[2], Claude Desplan[1] [1]Biology, New York Univeristy, [2]Biology, New York University

The identification and characterization of neuronal cell types in the optic lobe of Drosophila melanogaster is significant for unraveling visual processing mechanisms, with a potential understanding of similar processes in humans. There are over 200 different neuronal cell types within the fruit fly's visual circuit. To understand individual neuronal function and development, the generation of cell-type-specific genetic tools for labeling neurons is an essential first step. Prior studies utilized active enhancer-GAL4 lines to label diverse cell types. However, the limited specificity of the tool prompted the development of an innovative approach. The split-GAL4 lines take advantage of the intersectionality of the cells to refine the cell-type-specific expression; however, these enhancer-based split-GAL4 lines require time and resources to screen all the different enhancer patterns. In this study, we utilize the single-cell RNA sequencing (scRNAseq) of all the individual cells in the optic lobe at different developmental stages to select gene pairs for the generation of gene-specific split-GAL4 lines. This novel technique shows effective labeling of both known and previously unidentified cell types within the Drosophila visual system. I evaluated the effectiveness of this technique by testing two distinct split-GAL4 lines targeting different cell types, guided by data derived from the developmental scRNAseq dataset. One split-GAL4 line tested was bi-DBD + Rx-AD, which is predicted to label cluster LC14. Another line tested was CG9896-DBD + Dop1R2-AD predictably labeled cluster 45, which is an unannotated cell type. The result of the studies showed that bi-DBD+ Rx-AD does label LC14 at three different stages tested. CG9896-DBD + Dop1R2-AD labels a TmY-like neuron from early pupal stage to adult. Our study showed the promise of cell-type-specific tools to effectively label established cell clusters but also unveiled the identity of unannotated clusters. This breakthrough methodology thus paves new ways for investigating the heterogeneous cell types within the optic lobe and providing further understanding of the visual system and its functioning. In the upcoming semester, I plan to create more Split-Gal4 lines and use MCFO and GFP staining to not only observe cell types in adult flies but also track their development across different stages of fly growth. We will be analyzing our data using confocal microscopy to assess the morphology and location of the neurons that are expressed with each generated split-Gal4 line.

1836S **Structure-function of *Drosophila* Robo3 using CRISPR gene replacement** Ayawovi Selom Ametepe[1], Abigail Carranza[1,2], Timothy Evans[1] [1]Department of Biological Sciences, University of Arkansas, [2]Texas A&M University School of Medicine

At the midline of bilaterians, multiple decisions involve neuronal extensions called axons. They are capable to sense chemical cues through transmembrane proteins named axon guidance receptors. One of the key components of axon guidance is the Roundabout (Robo) family. They are found in many animal groups, including insects and vertebrates, and they regulate many important axon guidance decisions. In Drosophila, there are three Robo receptors: Robo1, Robo2 and Robo3; all of them bind to a common ligand, Slit. The repellent ligand Slit, and its receptors regulate midline crossing of axons during development of the embryonic central nervous system (CNS). And each receptor plays a specialized role during development of the embryonic CNS Drosophila Robo3 has 5 immunoglobulin (Ig), 3 fibronectin type III repeats and 2cytoplasmic motifs. It is expressed in the lateral-most two thirds of the embryonic ventral nerve cord. It has been proposed that Robo3 regulates axon pathway formation in response to Slit, perhaps by conferring sensitivity to a Slit gradient emanating from the midline, but this hypothesis has not been directly tested. It is also unknown which other domains within the Robo3 receptor, if any, are required for its role in intermediate pathway formation. To determine which structural domains within the Robo3 receptor are required for its role in intermediate pathway formation, we used a CRISPR/Cas9-based approach to replace the robo3 gene with modified versions in which individual domains have been deleted. We generated variants of the Robo3 in which the N-terminal

Ig1 domain and Ig4 are deleted: we constructed CRISPR donor plasmids and a corresponding guide RNA plasmid to target the robo3 gene and replace it with our domain deletion variants. Drosophila flies expressing Cas9 were injected with the gRNA plasmid and robo3 variant donors, and we recovered the modified alleles by screening the injected flies' progeny via PCR. We observed that intermediate pathways failed to form properly in the variants lacking Ig1 and Ig4. This shows that Ig1 and Ig4 are required for proper localization of the Robo3 protein. We also found that in both knock-in allele that expression is not only limited to the two third lateral as it appears in the homozygote embryos and full length Robo3 protein, rather it is spread all over the axonal pathway. We further notice an abondance of HA tagged Robo3 lacking Ig1 and Ig4 respectively in neuronal cell bodies. This suggests that Robo3 Immunoglobulin domain Ig1 and 4 are essential for axonal localization of the Robo protein. We finally showed that Slit binds robustly to cells expressing full length Robo3 but does not bind to variants lacking Ig1. This suggests that Ig1 is required for Slit binding.

1837S **Multiple glial subtypes interact and compensate for the loss of nearby glial function** Gabby Salazar[1], Allison N Beachum[2], Kevin Krause[1], Amelia Nachbar[1], Hannah Klose[1], Hayes Miller[1], Jaeda Coutinho-Budd[2] [1]University of Virginia, [2]Neuroscience, University of Virginia

Glia not only form close associations with neurons throughout the CNS, but they also interact closely with each other. As these cells mature, they undergo glial tiling to neighbor one another without invading each other's boundaries. While glial tiling is a common phenomenon in the animal kingdom, little is known about the interactions that lead to and maintain this process. Through an RNAi-based screen in *Drosophila*, we discovered that the loss of the secreted neurotrophin Spätzle 3 (Spz3) resulted in the transformation of cortex glia from a lace-like morphology that surrounds neuronal somas, to globular cells that lose the ability to ensheath these neurons. Our previous research revealed that the loss of Spz3 in cortex glia led to astrocytic processes infiltrating the cortex beginning in late larval stages. More recently, we observed similar outgrowth in other glial subtypes, such as ensheathing glia and subperineurial glia (SPG). Our developmental analysis demonstrated that cortex glia are initially established correctly, and only begin to exhibit altered morphology in late larval stages. Surprisingly, restricting animal growth prevented both cortex glial disruption and infiltration from other glial cell types even with the loss of Spz3, suggesting that this infiltration is not simply due to reduced Spz3 signaling but also the loss of cortex glial function as the CNS grows. We hypothesize that this aberrant infiltration is a compensatory mechanism of other glial subtypes for glial roles, such as clearing neuronal corpses throughout development. We therefore blocked engulfment in neighboring glia by reducing the engulfment receptor Draper in each subtype, and showed that astrocytes, ensheathing glia, and SPG contribute to the encapsulation and degradation of neuronal debris in the absence of functional cortex glia. Our data also demonstrate that peripheral phagocytes, such as macrophages, can infiltrate the CNS in these mutants. Remarkably, our findings suggest that even when astrocytes, ensheathing glia, and SPG divert their cellular resources from their normal positions, they are still capable of performing their usual functions. This work illustrates that when one glial subtype is disrupted and unable to perform its functions, surrounding healthy glia infiltrate beyond their natural barriers and perform compensatory actions alongside their normal functions to maintain CNS homeostasis.

1838S **Dissecting the Causal Role of Insomnia in Cardiovascular Disease** Torrey RP Mandigo[1], Farah Abou Daya[2], Suraj Math[3], Dev Patel[2], Lily Ober[2], Matt Maher[3], Girish Melkani[2], Richa Saxena[3], James Walker[3] [1]Center for Genomic Medicine, Massachusetts General Hospital, [2]University of Alabama at Birmingham, [3]Center for Genomic Medicine, Massachusetts General Hospital/Harvard University

Insomnia disorder occurs in 10-20% of the population and is an important risk factor for incident cardiovascular disease (CVD) conferring a >2-fold increased causal risk of incident. However, the underlying pathways and mechanisms linking the two remain poorly understood. We utilized the recent advances in large-scale sleep GWAS to identify human genes on haplotypes associated with insomnia and other sleep traits. From these candidate loci, we prioritized those with only one or two genes located within the haplotype and/or, with prior evidence of links to CVD, coronary artery disease (CAD), or cardiometabolic disease (CMD). After identifying *Drosophila* orthologs of these candidate genes, we used RNAi to knock down the expression of each ortholog in neurons to assess their cell-autonomous effects on sleep. Since mendelian randomization studies in humans have found insomnia symptoms causally contribute to CAD, we also looked at cell non-autonomous effects of neuronal KD on cardiac function and heart KD on sleep. These cell non-autonomous experiments highlight various pathways that are essential for the functions of both tissues as well as potential pathways conferring cell non-autonomous regulation of sleep and cardiac function, providing a shortlist of therapeutically relevant genes and pathways that link insomnia, sleep and CVD.

1839S **Postnatal normalization of *Dyrk1a* in a mouse model of Down syndrome impacts adult behavior and cognition** Elysabeth Otte[1], Drew Folz[1], Nicole Gordon[1], Charles R Goodlett[2], Randall J Roper[1] [1]Biology, Indiana University-Purdue University Indianapolis, [2]Psychology, Indiana University-Purdue University Indianapolis

Down syndrome (DS) is the most common genetic cause of cognitive disability, affecting approximately 1 in 700 individuals. DS is caused by the triplication of human chromosome 21 (Hsa21), leading to the overexpression of over 200 protein-coding

genes, including dual-specificity-tyrosine-phosphorylation-regulated kinase 1A (*DYRK1A*). *DYRK1A* catalyzes phosphorylation on serine/threonine and tyrosine residues and plays a role in cellular proliferation and neural development. Studies have linked *DYRK1A* to the cognitive deficits seen in individuals with DS, and further understanding the role of *DYRK1A* in cognitive development and functioning is crucial to understanding this disorder. We hypothesize that normalization of *Dyrk1a* beginning at postnatal day 0 (P0) will alleviate cognitive and motor deficits in a mouse model of DS. Using a DS mouse model with a temporally-activated *Dyrk1a* normalization, we are able to study the effects of disomic *Dyrk1a* expression in an otherwise trisomic animal. We tested these mice at P45 in a battery of behavioral analyses, including locomotion, gait, balance beam, and Morris Water Maze (MWM) to examine behavioral and cognitive function. Preliminary analyses indicate a significant increase in activity levels in female trisomic animals, and that trisomic females with two copies of *Dyrk1a* decreases this hyperactivity, but not to euploid levels. Male trisomic animals with a *Dyrk1a* normalization have similar activity patterns to those seen in euploid animals. Preliminary data show an increased step angle and slightly decreased total step distance in animals with normalized *Dyrk1a* levels when compared to euploid controls. Initial analysis of spatial learning and memory abilities of these animals show increased latency to locate visible platforms in trisomic females, and that trisomic females with two copies of *Dyrk1a* have latency values more closely resembling euploid counterparts. Normalization of *Dyrk1a* in an otherwise trisomic model seems to directly impact behavior associated with cognition and movement in a mouse model of DS, but does not rescue all deficits seen in trisomic animals. These data suggest that triplicated *Dyrk1a* may act in conjunction with other genes in the development of cognitive deficits in DS.

1840S **Expression Characterization of a Putative Novel Neuropeptide** Vicki J Wong, Jenny Chen, Sean Eddy Department of Molecular and Cellular Biology, Harvard University

Neuropeptides are signaling molecules that play a key role in the regulation of both behavioral and physiological processes, and it is believed that more neuropeptides remain to be discovered. We implemented a computational screen to discover putative novel neuropeptides and identified one gene that appears to be expressed and secreted in the brain. To understand the functional significance of this gene, we compiled and analyzed terabytes of publicly available RNA-sequencing data, with a focus on development and reproduction. The analysis sought to investigate expression patterns across species and pinpoint conditions associated with unregulated expression. To streamline these processes, an automated pipeline was developed, encompassing data scraping, read alignment, and expression calculation. We find this gene is expressed across vertebrates, specifically in the hypothalamus and pituitary. To experimentally validate these computational predictions, we plan to create knockout mouse models to understand the physiological consequences of its absence.

1841S **Antennal RNAseq reveals candidate genes associated with host preference in the Northern house mosquito *Culex pipiens*** Theresa N Menna, Katherine Bell, Anna Noreuil, Megan L Fritz University of Maryland

Culex pipiens, the Northern house mosquito, is the primary vector for West Nile virus (WNv) in North America. Its two distinct bioforms, *Cx. p. pipiens* and *molestus*, have divergent host preferences—*pipiens* prefers avian hosts, while *molestus* prefers mammals. However, hybrid offspring display indiscriminate host choice. Host preference dynamics crucially impact transmission rates for many vector-borne diseases, so it is therefore critical to identify the genetic underpinnings of this trait in *Culex*. In this study, we used a host landing assay to phenotype individual host-seeking females from three field-isolated strains of *pipiens* and *molestus* by their host preference. We then dissected and pooled antennae by phenotype for RNA sequencing in order to observe differences between human- and chick-seeking antennal transcriptomes. Our differential expression analysis focused on four chemosensory gene families recently annotated for *Culex*, known to play important roles in olfaction: odorant-binding proteins (OBPs), odorant receptors (ORs), ionotropic receptors (IRs), and gustatory receptors (GRs). Through comparative analyses between the antennal transcriptomes of human- and chick-seeking pools of *Culex*, we have identified 59 strong candidate genes associated with host preference, across 3 gene families known to detect host-specific odors. IRs from the understudied IR20a clade were strongly represented among differentially expressed chemosensory genes, indicating a promising avenue for future research. These results contribute to a deeper understanding of host preference genomics in *Culex* and lay the groundwork for future functional studies, moving us closer to improved vector surveillance for WNv in North America.

1842S **Paternal inheritance of SLC52A1 Gene Mutation in the infant presenting with developmental delay: a case report** Ah Yeon Lee[1], Hye Jung Park[1], Junghyeon Park[1], Myungshin Kim[2], Hoon Seok Kim[2], Joo Hyun Park[1] [1]Department of Rehabilitation Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, [2]Department of Clinical Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea

Introduction

Riboflavin, also known as vitamin B2, plays a critical role in the metabolism of various substances, such as fatty acids and

amino acids, serving as major energy sources. The body does not produce riboflavin, so any deficiency in its absorption can lead to pathological conditions. In severe cases, like riboflavin transporter deficiency (RTD), it can result in progressive neurodegeneration. RTD is associated with mutations in the SLC52A1 to SCL52A3 genes, each encoding a riboflavin transporter (RFVT1 to 3), leading to three different types of RTD (RTD 1 to RTD 3). While RTD2 and RTD3 are well-documented, RTD1 and mutations of the SLC52A1 gene are less common. Most cases are related to maternal inheritance due to the significant presence of RFVT1 in the placenta, affecting fetal riboflavin absorption.

Case Report

A 25-month-old male was born at term via cesarean section with a birth weight of 3600g following in vitro fertilization. At 15 months, he was referred to our rehabilitation medicine department due to developmental delay, despite no family history of developmental delay in naturally conceived siblings. Bayley-III Scales assessments at 15 and 25 months showed severe delays in all areas. An 18-month brain MRI revealed chronic periventricular leukomalacia, unmyelinated white matter, and thinning of the corpus callosum. Diffusion tensor tractography indicated that bilateral corticospinal tracts and the corticopontocerebellar tract from the left cortex to the right cerebellum couldn't be traced, explaining his severe gross motor delay and hypotonia. Genetic evaluation, including chromosomal analysis, chromosomal microarray analysis (CMA), and targeted next-generation sequencing (NGS), detected an SLC52A1 gene (c.1164_1165del) mutation. Since the mutation was of uncertain significance and RTD1 is less understood, segregation analysis was performed, identifying the same mutation in the asymptomatic father. This is intriguing as maternal inheritance is typically associated with RTD1 due to RFVT1 dominance in the placenta. Although the patient's symptoms do not align with typical RTD cases, his muscle tone reduction and impaired cognitive function may be related to a neuronal generation defect. As a result, the patient was referred to another clinic for further evaluation of riboflavin deficiency and potential treatment.

Conclusion

We presented a case of paternal inheritance of an SLC52A1 gene mutation in a patient with severe developmental delay, along with an asymptomatic father carrying the same mutation. While most RTD1 cases are associated with maternal inheritance, our case suggests that the paternal inheritance of the mutation itself may be the cause of developmental delay. However, given the limited knowledge about RTD1 and the SLC52A1 gene, further research is needed to fully understand this case.

Acknowledgements

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Examining the role of *cadherin 16* (*cdh16*) in the establishment of sensory thresholds Susannah S Schloss, Zackary Q Marshall, Nicholas J Santistevan, Jessica C Nelson Cell and Developmental Biology, University of Colorado Denver

An organism's ability to appropriately respond to environmental stimuli hinges on a precise combination of genetic and molecular factors that influence early neurodevelopmental processes. In particular, sensory thresholds, which dictate the level of stimulus required to elicit an animal's response, whether visual, auditory, or otherwise, are established during development. To explore sensory thresholds and their underlying genetic and circuit mechanisms, we use the acoustic startle response in larval zebrafish. Through a forward genetic screen, we identified a gene called *cadherin 16* (*cdh16*) as an important regulator of acoustic sensory thresholds and threshold plasticity through habituation. How *cdh16*, which encodes an atypical cadherin, exerts these functions is not yet known. To examine this question, we first assessed *cdh16* expression in the brain via *in situ* hybridization chain reaction. Next, we created a heat shock transgenic construct to test if ubiquitous expression of *cdh16* in mutant animals would rescue their sensitivity and habituation phenotypes. We found continuous expression of *cdh16* later in development, when the acoustic startle has been established, was sufficient to induce wild-type levels of responsiveness in mutants. Finally, we hypothesized based on a previous study that *cdh16* expression might be regulated by Wnt signaling, leading us to investigate the effects of pharmaceutical agonists and antagonists of Wnt-signaling on acoustic startle thresholds and habituation. We discovered that a Wnt agonist, XV, causes decreased sensitivity to acoustic stimuli, but has no impact on sensory thresholds in *cdh16* mutants. These data are consistent with *cdh16* functioning downstream of Wnt in the establishment and plasticity of sensory thresholds.

1844S **Identifying molecular targets of CHD7 that mediate CHARGE syndrome phenotypes in a zebrafish mode** Melody Hancock[1], Dana Hodorovich[2], Rachael Bieler[1], Chris Cole[1], Kurt Marsden[1] [1]North Carolina State University, [2]National Institute of Environmental Health Sciences

CHARGE syndrome is a developmental disorder that affects 1 in every 10,000 births, and patients exhibit ear, eye, craniofacial abnormalities, and behavioral characteristics that include autistic-like behaviors, intellectual disabilities, and sensory

disorders. A de novo mutation in CHD7 (chromodomain helicase DNA binding protein 7) causes 70% of CHARGE syndrome cases. The Chd7 protein is a chromatin remodeler and has thousands of predicted binding sites in the genome. Our lab created a zebrafish model of CHARGE syndrome with a CRISPR/Cas9-induced 7 bp frameshift mutation in exon 9. This model recapitulates CHARGE-like phenotypes including craniofacial defects, ear defects, cardiac defects, and defects in both acoustically- and visually-driven behaviors. But the neural and molecular pathways that underlie these behavior defects are unknown. To determine the specific parts of the brain that require Chd7, we used brain-wide imaging to identify regions with altered activity patterns as well as altered numbers of inhibitory and excitatory neurons. Here we will show that, consistent with our behavioral data, chd7 mutant larvae have reduced activity in hindbrain areas required for specific responses to acoustic stimuli. We also show that chd7 heterozygotes have an increase in inhibitory neuron labeling in this same region. To identify Chd7 target genes that mediate CHARGE-like behavioral phenotypes, we are currently analyzing transcriptomic and proteomic data from chd7 wild type, heterozygous, and homozygous mutant zebrafish brain tissue at two developmental time points. We are using differential expression analysis, ingenuity pathway analysis (IPA), and machine learning approaches to define a set of strong candidate genes that link Chd7 with disease-related phenotypes. To determine if the identified pathways impact CHARGE-like phenotypes in our zebrafish model, we will design CRISPR gRNAs to knockdown or enhance expression of candidate genes and measure morphological and behavioral phenotypes and assess whether they phenocopy or rescue CHARGE-like phenotypes. Together these analyses will bridge a key gap and allow us to define molecular and cellular pathways that may be targets for potential therapeutic intervention to alleviate specific aspects of CHARGE syndrome.

1845S **Serotonin acutely regulates acoustically-evoked behavior selection in zebrafish through multiple HTR2 receptor subtypes** Rebecca Osbaldeston[1], Kevin Villafañe[1], Matthew Curran[1], Nicholas Roland[1,2], Eva Shin[1], Roshan Jain[1] [1]Biology, Haverford College, [2]Neurobiology and Behavior, Cornell University

Neuromodulators such as serotonin (5-HT) regulate many aspects of behavior including mood, social interactions, sleep, and decision-making, to allow animals to flexibly respond to their changing environment. We focus on simple acoustically-evoked behavioral selection in zebrafish to model and understand the impact of serotonin on decision-making and behavioral flexibility. Following sudden acoustic stimuli, zebrafish larvae select between two stereotyped escape behaviors: an explosive short-latency response or a kinematically distinct and weaker long-latency response, biasing their selection based on the perceived threat of the stimuli, environmental context, and recent stimulus history. Through an array of pharmacological antagonists and agonists of various serotonin receptors, we have identified distinct serotonin receptor subsets that acutely bias which escape behaviors zebrafish select in response to acoustic stimuli. To pinpoint the specific serotonin receptors responsible for biasing this behavior selection, we designed CRISPR/Cas9 gene knockout tools to target candidate serotonin receptor subtype genes. Through combined pharmacology and genetics, we demonstrate that 5-HT_{2B} and 5-HT_{2CL2} receptor activity promotes the selection of long-latency escape behaviors over short-latency responses. Surprisingly we also find that the related paralogous 5-HT_{2CL1} receptor biases behavior selection in the opposite direction, promoting short-latency escape behavior. To identify the neuronal changes underlying these functions, we have begun mapping whole brain neural activity changes using the photoconvertible CaMPARI2.0 sensor. Together this work reveals multiple 5-HT receptor mechanisms through which serotonin bi-directionally modulates simple and ethologically relevant vertebrate decision-making following acoustic threat.

1846V **Characterization of Ultrasound-Induced Mobility Defects and Subsequent Recovery in *C. elegans* Nematode Worms** Louise M Steele[1], Monica Flores[2], Caitlyn McTrustry[2] [1]Biological Sciences, Kent State University at Salem, [2]College of Nursing, Kent State University at Salem

Our lab has reported that exposure to therapeutic ultrasound reduces *C. elegans* worm mobility in a dose-dependent manner. As the ultrasound intensity was increased, fewer worms were thrashing normally, and more worms exhibited a slow, irregular movement that we called «writhing» (Steele *et al*., 2021). At the higher intensities tested, some worms became immobile. To characterize the worms' mobility defects in more detail, we analyzed their movement in S. Basal buffer immediately after ultrasound exposure by using WormLab software. Depending on the severity of the defects, the worms' average and maximum body bend angles were reduced, and different curvature map "signatures" were often observed. Interestingly, many writhing worms appeared to recover their mobility within minutes or hours. To assess whether they made a full or partial recovery, we determined their thrashing rates by using the free ImageJ plugin, wrMTrck. After three hours of recovery time, their average thrashing rate was the same as that of unexposed shams, which suggested that their mobility was fully restored. To explain the transient nature of the defects in movement, we hypothesized that ultrasound may alter neurotransmission. Indeed, writhing worms were hypersensitive to aldicarb, which suggested that acetylcholine signaling was affected. To rule out the possibility that writhing worms were simply more permeable to aldicarb, we stained them with SYTOX Green, a fluorescent dye that only enters cells with compromised membranes. Based on those results, at least some writhing worms were unlikely to be taking up aldicarb more readily. Future work will determine if the neurotransmission defect is pre or postsynaptic. This study has the potential to deepen our understanding of ultrasound's bioeffects in the nervous system at the cellular and molecular levels.

This information could contribute to the development of exciting new laboratory techniques and therapeutic procedures.

1847T **Creating a course based undergraduate research experience (CURE) genetics laboratory course at Xavier University of Louisiana.** Joanna E Haye-Bertolozzi Biology, Xavier University of Louisiana

Course-based Undergraduate research experiences (CUREs) are important for providing undergraduates with authentic research experiences. We developed a CURE course for an undergraduate Genetics Laboratory at Xavier University of Louisiana. The goals of developing this Genetics CURE laboratory course were: 1) to provide an opportunity to participate in a hypothesis driven research project for a large number of students, 2) to analyze different alleles of *MSH6* and determine the effect of each variant on DNA mismatch repair (MMR), 3) to provide opportunities to develop scientific writing skills, and 4) to assess student learning. Our long-term goal is to assess the impact of the CURE course on student confidence, interest in science, and persistence in STEM. Examination of course evaluations indicated that students appreciated working on a single project for the semester and gained understanding of the effect of different mutations on the DNA MMR process. Analysis of pre- and post-course surveys demonstrated an increase in student sense of belonging in the field of science and perception of themselves as scientist.

1848T **Theory, Design, and Outcomes for routine DEIJ discussion during Department Meetings** Katherine Furniss[1], Catherine Kirkpatrick[1], Bhaskar Upadhyay[2] [1]Biology Teaching and Learning, University of Minnesota - Twin Cities, [2]Organizational Leadership, Policy, and Development, University of Minnesota - Twin Cities

Undergraduate institutions are increasingly asking their employees to engage in Diversity, Equity, Inclusion, and Justice (DEIJ) workshops, committees, and programs. Some open questions are which DEIJ efforts will lead to meaningful change in participant attitudes and actions, are scalable, and align with participant interests?

Beginning Fall 2021, the Biology Teaching and Learning (BTL) Department at the University of Minnesota-Twin Cities has engaged in a monthly exploration of a variety of DEIJ topics through the framework of transformative mindfulness. During the planning stage, BTL department members and administrators analyzed change theories to design an approach that: 1) would influence and communicate departmental culture, 2) factored in time limitations for department members, and 3) employed evidence-based practices around individual and community change. The designers based the program goals on the principles of self-awareness and empathy laid out in Dewsbury and Brame's 2019 article on Inclusive Teaching. Over the course of two years and 12 department meetings many DEIJ topics have been discussed including meritocracy, color blindness, achievement vs opportunity gap, and Relational Cultural Theory.

After two years, we wanted to know whether there has been any meaningful change to self-awareness and empathy related to DEIJ concerns, attitudes about departmental culture, or behaviors. Using a theoretical framework by Morris 2020, Incremental Transformative Learning Process Through Mindfulness, we analyzed data from department meeting artifacts (anonymous open-ended and quantitative surveys, jamboards) as well as semi-structured interviews with department members. We have found evidence that the department meeting program interrupts the connection between an individual's frame of reference (how they perceive themself and the world around them) and their behavior (thoughts, judgements, feelings, and actions). We also found evidence that department members recognized their implicit motivational dispositions and that some of them changed their behavior. We will discuss potential modifications to the framework, lessons for implementation in other environments, and project limitations.

Dewsbury and Brame. 2019. doi: 10.1187/cbe.19-01-0021 Morris. 2020. https://files.eric.ed.gov/fulltext/EJ1257177.pdf

1849T **Do I Belong? An Analysis of Black Student Experiences in the Biology/STEM program at Emmanuel College** Samira Fawel[1], Jennika Fevrier[2], Anupama Seshan[1] [1]Biology, Emmanuel College, [2]Emmanuel College

A Belonging in Biology initiative was undertaken by an undergraduate Black-identifying Biology/Health Sciences major at Emmanuel College (EC), a primarily white institution (PWI). Current biology majors and recent alumni from the EC Biology program that also identified as Black were invited to share their experiences navigating the Biology and Biology-related STEM programs. A total of ten interviews were conducted with nine female-identifying, and one male-identifying student. Three participants were alumni of Emmanuel while seven were current Emmanuel students at the time of the interview. Semi-structured interviews were conducted and coded using the Taguette open-source tool. The codes revealed a total of 17 major themes, with eight themes related to exclusion, and nine related to inclusion. The frequency of exclusive codes (199) far outnumbered the frequency of inclusive codes (91). The most frequent exclusive themes mentioned were 1) lack of diversity in the classroom and on campus (n=53) and 2) lack of discussion about student's self-identity and current issues (n=39). The most frequent inclusive themes included 1) participating in established programs created specifically for students of color (n=18) and 2) feeling supported through Black students' and peers' presence and interactions (n=16). A compilation

video was created to highlight the most frequent exclusive and inclusive codes and shown to School of Science and Health (SSH) faculty at Emmanuel College during a professional development workshop. Following the viewing, faculty participants demonstrated increased awareness of major reasons why some students are or are not successful in STEM fields. For example, 59% of participants noted that lack of belonging in STEM courses is a major reason pre-workshop, but this shifted to 83% post-workshop. Pre-workshop, 53% of participants believed that lack of sufficient knowledge in math and/or science was a major reason, while post-workshop this dropped to 25%. Overall, our study presents a set of themes to explain factors that prevent minoritized undergraduate students at PWIs from persisting in their chosen field, as well as those that increase persistence. We investigate how participating in initiatives to raise awareness about systemic injustice increases belonging for minoritized students. This research demonstrates a model for how similar institutions can learn from their own students and share student experiences to change faculty mindsets.

1850T **The Basics of NIH Peer Review** Mollie Manier, Guoqin Yu National Institutes of Health Center for Scientific Review

As the largest funder of biomedical research in the world, NIH supports much research relevant to scientists at TAGC. NIH funds are competitively awarded through a two-stage review process. The first level of review solely evaluates the scientific merit of an application by convening peer reviewers who are experts in the field to adjudicate the potential impact of the proposed research. The second level of review takes into consideration for funding both the scientific impact of a proposal as well as its responsiveness to NIH programmatic objectives. The majority of peer review takes place through the NIH Center for Scientific Review (CSR). CSR conducts the review of 94% of R01s, 84% of NIH NRSA Fellowships, and 96% of SBIR-STTR applications. Outreach efforts by CSR scientific review officers help investigators and reviewers understand important aspects of the grant submission and review process. Key areas for discussion cover the basics of peer review, review criteria, the review timeline, whom at NIH to talk to about your application and when, how to find a potential study section, and recent policy initiatives, such as those pertaining to simplified review criteria, data management plans, and peer review integrity. Outreach efforts help to minimize the influence of grantsmanship and differential knowledge of NIH priorities, policies, and practices, to ensure that review outcomes reflect the strength and scientific merit of applicants' ideas.

1851T **Development of an Oxford Nanopore CURE module for an undergraduate genetics course.** Bryce Taylor Program in Biology, Loras College

It is critical for today's genetics students to gain exposure to genomic tools like next-generation sequencers. Unfortunately, these tools are generally out of reach for most small colleges. The MinION sequencing devices from Oxford Nanopore offer a relatively accessible entry-point for genomic investigations, but to date there are few models of how to incorporate these into classroom instruction.

In my genetics course we are using Minion sequencers to sequence microbial genomes during 4-week CURE modules. I have followed two models for this module:

1. Student groups submit project proposals for consideration. All students participate in sample collection and analysis of data generated from one MinION Mk1B flow cell.

2. I pick a project and have each student collect and sequence samples on individual MinION Flongle flow cells.

I'll report qualitative perspectives on how both models went, data generated from both years, and challenges faced and overcome in developing this CURE module at a low-resourced institution. I would appreciate feedback and perspective from other educators or minion users!

1852F **Structures and Outcomes of Virtual Human Genomics and In-Person *Caenorhabditis* Genetics CUREs** Sosse Kendoyan[1], Joseph Ross[2] [1]California State University, Fresno, [2]Biology, California State University, Fresno

The inclusion of research within the undergraduate academic curriculum is advantageous to students, in part by democratizing access to research opportunities for students. Thus, we redesigned the upper-division undergraduate course Cell Biology and Genetics laboratory (BIOL 104) as two Course-based Undergraduate Research Experiences (CUREs). Here, we share the course designs and their student impacts. Students in the in-person sections developed and conducted *Caenorhabditis* genetics research projects. They first trained in laboratory techniques like microscopy, worm husbandry, crossing, and PCR genotyping. Small groups of student teams then conducted a scaffolded literature review, generated a hypothesis and experimental design, collected and analyzed data, and interpreted and presented their work over the course of a semester. Each group prepared a written reports in the format of a *Micropublication Biology* journal article, so that any projects that support a novel hypothesis or fail to replicate published literature can be easily submitted for peer review and possible publication. Some groups were also selected for oral presentations at a campus research symposium. Student surveys indicated support for this approach.

Students suggest that such research courses be offered earlier in the curriculum and that the potential for publication sustains motivation in course-based research. Our findings suggest useful ways to design CUREs that address issues with resource availability (e.g. time, facilities, and funding), student perceptions of the value of CUREs, and useful approaches for assessing student gains. We also suggest ways to balance student training in knowledge and practical content while providing as much time as possible for their research. Students in the virtual section learned to interpret human ancestry testing datasets. Each student group identified a point mutation that influences a human trait and that is genotyped by ancestry testing companies. The project goal was to research that mutation and to draft a report to the anonymous donor, based on their genotype, predicting their trait or disease risk. Custom videos provided instruction on how to use SNPedia and OMIM and to interpret the raw genotype data files. Students found this approach to be engaging and practical by providing them with knowledge and skills they can apply to their own lives. Students also highlighted how the course contributed to their academic and career aspirations.

1853F **Fly-CURE and connecting curriculum: multi-institutional course-based undergraduate research experiences in genetics and beyond** Kayla Bieser[1], Jacob Kagey[2], Julie Merkle[3], Jamie Siders[4], Joyce Stamm[5], Alysia Vrailas-Mortimer[6] [1]Nevada State University, [2]University of Detroit Mercy, [3]Biology, University of Evansville, [4]Ohio Northern University, [5]University of Evansville, [6]Oregon State University

The Fly-CURE is a multi-institutional course-based undergraduate research experience (CURE) centered on the genetic mapping and characterization of *Drosophila melanogaster* by undergraduate students at 26 institutions (including public, private, community colleges, and minority-serving institutions). To date, undergraduate researchers have successfully mapped and characterized 26 EMS-induced mutants, which has led to local and national scientific presentations by students, as well as eleven peer-reviewed publications with 581 undergraduate co-authors. This project has provided research exposure to greater than 1,500 undergraduate researchers within a classroom setting and student participants report significant gains in their sense of belonging to the scientific community, self-efficacy in research methods, and intent to pursue additional research opportunities. We are expanding the Fly-CURE curriculum through an NSF-funded Research Coordinated Network (RCN) to develop courses in bioinformatics, behavioral genetics, molecular biology/CRISPR, and developmental biology, which can be scaffolded with the genetics Fly-CURE modules or can be implemented as stand-alone CUREs. Through this RCN, we will increase research exposure for students across different courses and provide more opportunities for faculty to incorporate CUREs at their institutions. We are currently recruiting faculty to participate in our RCN. Faculty participants will be provided stipends for curriculum training and implementation, a social network of faculty, a community of like-minded scientists, continued scholarship opportunities, and support for tenure and promotion.

1854F **Inclusivity of the LGBTQ+ community in Emmanuel College biology courses.** Teagan Santoro[1], Janel Cabrera[2] [1]Biology, Emmauel College, [2]Biology, Emmanuel College

Teaching a curriculum that is inclusive and representative of diverse groups of people can result in higher outcomes for students and reduced stigma. However, LGBTQ+ discrimination and lack of inclusion has been growing across the US, and queer representation and education in schools tends to be scarce. While various studies have been conducted, there is little information available regarding implementing LGBTQ+ inclusive curricula, and this is particularly true in STEM fields. This research surveyed students and instructors in biology courses at Emmanuel College in Boston, MA to examine feelings of classroom and curriculum inclusivity on the basis of gender identity and sexuality. In the initial surveys, instructors were asked to answer questions regarding knowledge and understanding of LGBTQ+ terminology and topics, levels of comfort with discussing such topics with students, and personal efforts to make an inclusive curriculum and classroom environment. Students were asked about previous experiences of belonging and representation on the basis of gender identity and sexuality in classrooms, including Emmanuel biology classrooms, and personal desire to learn more about LGBTQ+ topics relating to their science courses. It is hypothesized that the current curriculum is markedly less inclusive towards those in the LGBTQ+ community and identifying students would report feeling less included and represented in the classroom environment than their heterosexual, cisgender peers. Based on the data received after the second set of surveys, which will be more focused on experiences of belonging and inclusion in the given biology course throughout the semester, resources and any necessary further actions will be compiled and planned for instructors to aide in development of more inclusive curricula. The goal is that the information gained through this research will provide a more open understanding of what must change in classroom planning and environment so students in the LGBTQ+ community feel sufficiently represented in material and can cultivate a better sense of belonging. While this initiative is currently localized to the biology department at Emmanuel College, the hope is to expand such research and resources to other departments and institutions.

1855F **Learning genetics by “making a baby” with a deck of cards for majors or non-majors courses** Tina L. Gumienny, Lionel L. Faure Biology, Texas Woman›s University

Genetics can be a difficult topic to master. For some students, the random yet precise segregation of chromosomes during

meiosis and different inheritance patterns are especially hard to grasp. To help students understand these basic genetic concepts, we developed, implemented, and refined a “card baby” active learning activity over eight years. This activity can be done in class or online, and in a non-majors or majors course. After instruction on meiosis, the animal life cycle, and basic inheritance patterns, students get to apply what they learned by “making a baby”. In-class students pair and are given a deck of cards. Each card represents a chromosome. The two black suits are the dad’s two chromosome sets. The two red suits are the mom’s two chromosome sets. Students are instructed to perform meiosis: after laying out cards in order by suit, one student in the group makes the haploid “sperm” chromosome set by randomly selecting one of each card number from clubs and spades. In a similar way, another student makes the “oocyte” chromosome set from heart and diamond cards. The group performs “fertilization” by matching up the two sets of cards. They confirm no aneuploidies. After students have successfully “made a baby”, they get a table with the genotypes associated with each chromosome and the inheritance pattern. Real human traits that generally follow autosomal recessive or dominant, sex-linked, incomplete, and polygenic inheritance patterns are provided for the students to interpret. The majors course includes an example of epistasis. The students’ goal is to interpret the genotype and phenotypes of their “baby” based on the randomly selected card chromosomes their “baby” has. They fill in a paragraph describing their baby’s traits based on the genotype and the given inheritance patterns. At the end of the class (or in a discussion board, if online), groups read their paragraph to the class so students can see the variety in the “siblings” the class made. Students provided overwhelmingly positive responses when asked if this activity helped them understand the patterns of inheritance and if this activity was enjoyable. This activity is adaptable to one’s favorite inheritance patterns, traits, and diploid, sexually reproducing, multi-chromosomal organism. Practically, it is inexpensive, doable in a 50- or 80-minute class, and scalable to large classes (especially with help from teaching assistant(s)).

1856F **‘Genetics & Society’: a non-majors undergraduate case study examining a genetic research study for scientific merit and societal impacts** Nicole M Green Biology, Cornell College

The field of genetics has produced many exciting leaps in knowledge and technology in recent years. However, rapid scientific advances place a high burden on individuals in society to follow current research trends and understand new technologies. In an effort to prepare college students about the role of genetics in their own lives and communities, I designed a non-majors course emphasizing critical skills such as inquiry, source evaluation, decision-making, and intercultural literacy. This course—called Biochemistry & Society—was developed within a unique educational model called One Course At A Time, which has students participate in a single class for 18 consecutive days. The flexibility of our course design creates an exciting pedagogical opportunity to deeply explore and engage with scientific topics.

A large section of Biochemistry & Society class was dedicated to the intersection of genetics and society. Our genetics module opened with discussions on the clinical trial process, the evolution of medical ethics, and the biomedical research pipeline. These foundations in scientific practice were followed by a historical perspective on the field of genetics and the future promises and challenges presented by recently developed genetic technologies such as CRISPR/Cas9 editing. The genetics module culminated in a case study that asked students to use skills from prior sessions to make a decision about participating in a current genetics research study at a local R1 institution. The genetic research study we examined aims to identify the complex genetic traits of giftedness and the phenomenon of twice-exceptionality. We built an investigative ‘file’ containing the email study invitation, intake survey, and study consent form. Furthermore, students were directed to the research lab website, previous publications, and press releases on current and past projects. Students then completed a self-guided assessment of the study materials and made an initial decision about participation. The self-guided activity was followed by a class-wide discussion to highlight good study design, identify areas of concern, and points of clarification before consenting to participate. Students were also asked to consider possible research applications and implications by reviewing media about the historical and ethical considerations of intelligence research, as well as a new movement in behavioral genetics attempting to bridge genetics and social justice initiatives. After many rounds of individual and group feedback, students were asked to reflect on their decision and how their perception and understanding of genetic research changed as a result of engaging with a specific research study. Student feedback following this case study highlights the importance for practical applications of genetics concepts and for building more curricula training non-scientists to engage with genetic research.

1857F **Implementing Equitable Grading in Microbiology** Suparna Chatterjee New Mexico State University

Assessment is an important criteria for measuring student success and specifications grading implemented in a microbiology course to assess student success in an equitable system. It is a grading system based on the mastery of specific educational outcomes that determine the final grade a student can earn in a course. I have designed a specifications grading strategy for the undergraduate Microbiology for Health Sciences course, creating 16 individual learning outcomes (LOs). Most of the students in this course are Nursing majors for whom this course is mandatory. It implemented two design principles: (i) detailed feedback on completed work, what is done well, and what needs improvement through practice or review, and (ii) additional opportunities to practice skills that are challenging but can be achieved by resubmitting assignments. The grade

earned in the lecture depended on the number of LOs the students mastered. A student's final class grade depended on the number of LOs mastered combined with the grade earned in the final exam. Implementing this grading system it was found that students showed positive attitudes towards learning microbiology and the DFW (students receiving D, F, and withdrawal) rate dropped.

1858F **Integrating Research Ethics Instruction in a Genetics CURE** Joseph Ross[1], Sosse Kendoyan[2] [1]Biology, California State University, Fresno, [2]California State University, Fresno

Course-based Undergraduate Research Experiences (CUREs) provide an opportunity for students to conduct the entire scientific method, from background reading and hypothesis generation to experimental design, data collection and analysis, and dissemination of results. In addition to these common CURE components, there is significant value in training students to be ambassadors of the objectivity and trustworthiness of scientific research to their communities. Thus, education in the responsible and ethical conduct of research (RECR) should be integrated with CUREs, so that students learn about and apply the ethical norms of science in the context of conducting research. In the redesigned Genetics and Cell Biology Laboratory CURE, we introduced direct instruction on the concept of research ethics and on five core research ethics topics defined by the US Department of Health and Human Services: recordkeeping, misconduct and fraud, authorship, plagiarism, and human subjects research. We selected these topics because of their direct relevance to student research projects, because students maintain research notebooks, analyze and manipulate data, produce co-authored works that require citations, and are human subjects participants in the present research study on this CURE design. We conducted pre- and post-semester surveys and assessed the impact of the training on student understanding and implementation of ethical processes and decisions in their CURE projects. We find that most students have never experienced a CURE course or participated in mentored research with a faculty member, and most have poor understanding of the relevant ethical norms at the start of the course. By the end, student performance improved in understanding the norms (e.g. they are able to provide a definition of plagiarism and describe why avoiding plagiarism is important) and also in applying that knowledge. For example, when presented with a passage from a published peer-reviewed manuscript (with the citations removed), students improved in their ability to identify which sentences should contain citations and to explain their decisions. Here, we share these qualitative and quantitative data and describe the research ethics curriculum and how it was integrated into the course, with suggestions for others who also want to introduce RECR training in their CURE.

1859F **Incorporating information literacy into genetics undergraduate curriculum** Elizabeth J Wade Natural Science and Math, Curry College

Information Literacy is the ability to access, evaluate, and use information from a variety of sources, which can be difficult for undergraduate students today given the incredible amount of information and misinformation available. Here, I discuss how to incorporate information literacy into an undergraduate genetics course for science majors using class discussion, current events, research articles and specific assignments. This also increases student engagement, deepening their understanding of genetic concepts and fostering a meaningful learning experience by focusing on topics that are relevant to students' lives and prior understanding.

1860F **Building Sustainable Approaches to Ethical Inclusion of Indigenous Peoples in Genomic Research** Julie Beans[1], Jessica Blanchard[2] [1]Research Department, Southcentral Foundation, [2]University of Oklahoma

This presentation will provide an overview of three areas where our research team aims to advance the ethical inclusion of American Indian and Alaska Native (AIAN) peoples in genomic research through: 1) balancing Tribal community priorities with collective goals; 2) distributing power in ways that promote equitable partnerships; and 3) providing research and training experiences to support AIAN student interest in genomics. This work is conducted as part of the Center for the Ethics of Indigenous Genomic Research (CEIGR), a multidisciplinary consortium led by a mix of community-placed tribal partners, university researchers and community-based institutions working collaboratively to conduct research on ethical, legal, and social implications of genomics in AIAN communities in the United States. CEIGR aims to model meaningful community engagement in genomics research and move toward inclusive and equitable research practices – central to our efforts is a shared perspective that community engagement requires community building. An important goal of CEIGR is to increase the number of AIAN scientists in the field of genomics by creating meaningful experiences for AIAN undergraduate students and extend a network of support for AIAN student and scientists to collaboratively advance the field. The empiric and normative work we present provides examples of how to build sustainable approaches to ethical research with Indigenous communities.

1861S **Using *C. elegans* to study *dcaf-13* RNAi phenotypes to promote inquiry-based learning in an undergraduate Cell and Molecular Biology course** Jessica Sullivan-Brown, Jessica Sowa Biology, West Chester University

Inquiry-based undergraduate laboratories are designed to introduce students to the excitement of making novel scientific

discoveries and perform independent research. Our objectives were to provide undergraduates with opportunities to gain genuine experiences in contributing to fundamental scientific knowledge by characterizing novel RNAi phenotypes of relatively understudied genes in the worm *Caenorhabditis elegans*. In our efforts to create these experiences, we designed labs for an upper-level Cell and Molecular Biology course at a primarily undergraduate institution in which students characterized the RNAi phenotypes of the potential oncogene DCAF13 (DDB1 and CUL4 associated factor 13). We discovered that when *dcaf-13* is disrupted in *C. elegans*, the worms showed a significant effect to larval development as measured by determining overall length of worms at 48 hours of development. In addition, *dcaf-13*(RNAi) worms display either a failure or delay of reaching the L4 or adult stages. Our data also indicates that *dcaf-13*(RNAi) did not affect embryonic development in *C. elegans*; however, egg-laying was significantly decreased in *dcaf-13*(RNAi) worms suggesting a general role in fertility. We were able to publish this work in *microPublication Biology*, with student authors from the Cell and Molecular Biology course and continue to use this study as a platform for novel discoveries.

1862S **The Genomics Education Partnership: A Path to Classroom Research Experiences in Person or at a Distance** Alondra M Diaz-Lameiro[1], Martin G. Burg[2], Wilson Leung[3], Jennifer Kennell[4], Chinmay P. Rele[5], Katie M. Sandlin[5], Stephanie Toering Peters[6], Laura K. Reed[5] [1]Biology, University of Puerto Rico at Mayaguez, [2]Grand Valley State University, [3]Washington University in St. Louis, [4]Vassar College, [5]The University of Alabama, [6]Wartburg College

The Genomics Education Partnership (GEP) is a nationwide collaboration of 271 faculty from 215 institutions that has impacted more than 12k students since 2018. Our main goal is to increase accessibility to genomics and bioinformatics through the incorporation of Course-based Undergraduate Research Experiences (CUREs). Participating institutions range from Community Colleges, Primarily Undergraduate and Minority-Serving Institutions, to Research Universities. GEP provides a web-based platform (thegep.org) with curated curriculum and training materials that can easily be incorporated into existing courses, a robust network of nationwide virtual TAs, and ample professional development before, during, and after curriculum implementation for faculty. During the COVID-19 pandemic, GEP curriculum proved to be an outstanding tool to continue student research experiences, which produced novel data, in an online framework. Through the GEP curriculum, students learn to annotate genes in eukaryotic genomes and become familiar with various bioinformatics tools. In the process they learn to leverage evidence from reference genomes, experimental data, gene prediction algorithms, evolutionary conservation, and molecular biology rules to create a defendable gene model. All without the need of external funds, expensive reagents, or equipment, making the curriculum and the authentic research experience accessible for smaller institutions, newer faculty, and/or online students. The GEP research projects include investigations on venom evolution in parasitoid wasps, evolution of insulin pathway genes across 31 *Drosophila* genomes, the F Element in four *Drosophila* species, and genes associated with egg formation in the critically endangered Puerto Rican parrot, with a handful of new faculty-led projects currently in development. Student gene models are reconciled to generate large datasets for genomic evolution studies. In addition, GEP supports publication of gene models as microPublications, with students as lead authors. The GEP also engages in science education research. Our most recent study found that Community College (CC) student gains were comparable to non-CC students, with improvements in attitudes towards science and thriving in science, making our model widely applicable. In addition to accessing the curriculum materials and tools, GEP faculty benefit from the support of a national network of colleagues. The GEP is supported by NSF IUSE-1915544 and NIH IPERT-R25GM130517 to LKR.

1863S **A cohort-based research program for early-career undergraduates** Elyse Bolterstein[1], Samantha Brown-Xu[2], Ignatius Gomes[3], Jennifer Hasso[4], Scott Mayle[5], Shreya Patel[4] [1]Biology, Northeastern Illinois University, [2]Chemistry, Northeastern Illinois University, [3]Harold Washington College, [4]Northeastern Illinois University, [5]Physics, Northeastern Illinois University

It is well known that participating in undergraduate research prepares for their future careers. However many research internships are designed for upper-level students, which limits participation of students still deciding on their career tract. To help bring research opportunities to students earlier in their academic career, we developed STEM Academy, which is an 8-week collaborative research program designed for first and second-year students who are interested in STEM fields, but have little to no formal STEM experience. Our goal for this program is to serve as a bridge to mentored research programs, internships, and STEM careers.

The STEM Academy fulfills a goal of the Advancing Careers and Research Opportunities (ARCOS) program at NEIU, a title III HSI STEM grant funded by the Department of Education. ARCOS brings together our institute with a local 2-year college (Harold Washington College) to enhance career development, research opportunities, and transfer efficiency to NEIU. STEM Academy was developed and implemented by a team of faculty representing various STEM disciplines to serve the interests of our students. Our inaugural student cohort consisted of 23 NEIU and Harold Washington students, with interests in biology, chemistry, environmental science, computer science, and psychology.

STEM Academy introduced students to lab techniques and experimental design through 2 collaborative research projects. First,

students worked in teams to explore the impacts of environmental contaminants and nutrients on Drosophila development and activity - a project that combined principles in biology, chemistry, and data science. Students then self-selected into teams mentored by STEM academy faculty to investigate research questions in their discipline of interest. For example, groups of biology students investigated diversity and antimicrobial resistance of microbes collected from waterways around Chicago. Throughout the program, students also participated in tutorials on common lab techniques (e.g. creating solutions, statistical analysis) and wrote weekly reflections on their experiences, designed to guide them in preparing application materials for internships, jobs, and graduate school. Students presented both projects to our group and university community. Overtime, we will track the impact of STEM Academy on students' sense of community, ownership, self-efficacy, and success in entry into future research programs and careers.

1864S **Rediscovering Mendel`s Developmental Genetics to Dissolve the Trouble of Gene-Centrism** Hui Zhang, Yafei Guo, Jiaqi Zheng, Wenli Zhao, Ling Wang, Fuli Ma Northwest Normal University of China

There is an increasingly explicit critique of the belief of gene-centrism or genetic determinism in the community of genetics educators. Also, a similar tendency appears that taged Mendel as enemy of genetics No. 1, accusing his first holding oversimplified idea of "gene for green/yellow pea seed" with no consideration for environmental effect in organism development. In history, genetics discipline was founded by the later rediscoverers, what was to Mendel?

At the bicentennial of Mendel's birth, we reconstructed Mendel's narrative regarding the selfing reproduction of hybrid by using his own symbol system, $Aa \times Aa = (A + a)(A + a) = A/A + a/a + A/a + a/A = A + 2Aa + a \rightarrow 3A + a$. Remarkably, the three equal signs in the sequential expression were respectively lectured by Mendel himself as the principles of reproductive cells formation, of fertilization, and of seeds developments in 1865. The same pair of symbols, *A*, and *a*, contains three different senses, gametes, factors, and traits, whose numerical corresponding relationships between two entities of the three were often expressed, but no word like"*A*/*a* gene for *A*/*a* trait" founded in the context. According to preserved Mendel›s letter to Nageli In 1870, one factor carried by one gamete with capacity to transmit one trait to offspring was eventually exemplified in his controlled pollination experiment in *Mirabilis jalapa*.

In fact, as a physics teacher, Mendel in his mind proudly regarded his core discovery as the development law of two constant forms (*A*, *a*) and one hybrid form (*Aa*) and published it as the sole mathematical equation in the paper, $A/A + a/a + A/a + a/A = A + 2Aa + a$. The left four fractions named "vivifying unions" are equal to but quite different from the genotypes in modern genetics, *AA*, *Aa*, *aa*. The latter is simplified as only "material composition of elements" while Mendel`s involving "their arrangement in primordial cell" as well. The fraction illustrating the intracellular spatial position effect of gene arrangement also implicates Mendel`s temporal meaning, the above from male gamete while the below female one. Besides the spatiotemporal environmental effect that Holliday defined as epigenetic control of gene expression, the inter-allelic, and the polygenic non-allelic interactions in foundation cells, as well as living conditions of organism in wild habitat were all taken into account in his paper.

Here we also report that Bateson, et al., might be responsible for the common mistake of gene-centrism, because they fragmented and oversimplified Mendelism as only part of transmission genetics but missing his most favorite developmental part. Sure, it is time to rediscover Mendel`s Mendelism to construct a complete and harmonious genetics.

1865S **Bridging Research and Education with Model ORganisms (BREWMOR)** Dondra Bailey[1], Kelli Carroll[2], Wen Chen[3], Sean Coleman[4], Rebecca Delventhal[5], Renee Geck[6], Paul Goetsch[7], Jill Keeney[8], Michael Law[9], Te-Wen Lo[10], Kelsey Perry[11], April Rich[12], Josefa Steinhauer[13], Bryce Taylor[14], Cindy Voisine[15] [1]Coppin State University, [2]Wofford College, [3]California Institute of Technology, [4]Wartburg College, [5]Lake Forest College, [6]University of Washington, [7]Michigan Technological University, [8]Juniata College, [9]Stockton University, [10]Ithaca College, [11]University of Colorado-Colorado Springs, [12]University of Pittsburgh, [13]Yeshiva University, [14]Loras College, [15]Northeastern Illinois University

BREWMOR (Bridging Research and Education With Model ORganisms) is an organization of educators and scientists united around our shared goal of building a network dedicated to increasing experiential learning for biology students, primarily at the undergraduate level. BREWMOR disseminates and propagates best practices to engage and support students in rigorous model organism-based research excellence while creating and fostering strong, diverse and inclusive communities. We want to connect with more researchers and educators at TAGC to exchange resources, knowledge, and experience, and expand our community.

BREWMOR was founded following the 2020 TAGC meeting, helping to fulfill a need for educators around the globe in delivering hands-on, discovery-based undergraduate learning experiences during the height of the COVID-19 pandemic. The original work of BREWMOR was focused on high-impact teaching practices that would engage students in an online learning environment. As such, BREWMOR's outreach has focused on the most pressing needs for educators.

We do this through hosting community-focused virtual workshops and seminars on topics at the intersection of research and education. These workshops have been sponsored by GSA and have generally fallen into two categories; a winter half-day workshop, microBREW, and a two-day summer symposium, bigBREW, where one day is a symposium and another day is a workshop. Previous event topics have included: DEI Basics for Science Educators, CUREs, Grants for Education-Related Work and Research at PUIs, and Integrating Primary Literature into the Classroom.

We are actively working on ways to maintain engagement between workshops, including peer groups, and provide opportunities for community members to report back on their experiences in learning new pedagogy and implementing these new approaches in their classrooms. Come visit our poster to learn more about our community-building endeavors and future workshops.

1866S **Teaching the Genome Generation: Cultivating High School Genomics Through Teacher Education** Erica L Gerace, Alexa Wnorowski, Christina Vallianatos, Sarah Wojiski, Charlie Wray Genomic Education, The Jackson Laboratory

Teaching the Genome Generation™ (TtGG) provides pre-service and current high school teachers with the content knowledge, teaching strategies, and resources needed to enhance student learning in genetics and genomics, with an emphasis on math skills and data literacy. Our approach weaves three learning strands—molecular genetics, bioinformatics, and bioethics—together within the context of the Next Generation Science Standards (NGSS) and Common Core Math Standards. Classroom study findings indicate use of our curriculum leads to increased knowledge of, and confidence in, biotechnology laboratory procedures and increased student interest in genetics and genomics concepts. Newly designed modules emphasize quantitative skills in four genetics- and genomics-related content areas: cancer genetics, sequence comparison and identity, genotype-phenotype associations, and ancestry. Each module includes standards-aligned activities focused on problem-solving or inquiry through the exploration, analysis, and interpretation of genetic and genomic data. Modules also encourage exploration of ethics and social justice topics relevant to modern genetics and genomics, such as health disparities and access to genetic testing. Our new content is freely available on our website and includes teacher guides and multimedia tutorials for new tools and skills. Recently, our resources have expanded to include a suite of laboratory simulations and interactives, hosted on LabXchange and available to teachers around the world.

1867S **LacApp: A platform to help students master gene regulation** Alex de Lencastre, Paul Wolujewicz, Caitlin Hanlon Biology, Quinnipiac University

The lac operon is widely taught to biology students as a paradigm for how gene expression is regulated from bacteria to humans. However, students struggle to correctly apply their understanding of gene regulation when prompted with higher order questions (Stefanski et al, 2016). We hypothesized that repeated self-testing, known as “retrieval practice” (Karpicke and Blunt, 2011), would help students develop a deeper understanding of these important concepts compared to traditional approaches such as lecture and worksheets. Here, we describe the design and initial testing of LacApp with quantitative and qualitative data. LacApp enables students to self-test using a series of randomly generated combinations of regulatory proteins, DNA binding sites, and genes from the lac operon to predict how a given combination of those elements would affect expression of the operon genes. Feedback indicating if the student’s prediction was correct is immediately provided along with an explanation of the regulatory logic. To assess the feasibility of LacApp, two sections of an introductory genetics course were divided into a control and experimental cohort. The control cohort completed a worksheet while students in the experimental cohort worked with LacApp. All students then completed a short multiple-choice quiz in class. Students using LacApp ($n=23$) scored significantly higher on the quiz compared to students who used a conventional worksheet ($n=22$; $p<0.05$). Following the study period, all students were given access to LacApp. Students indicated that LacApp was the most helpful learning source for understanding gene regulation and that the most useful feature in the app was the immediate explanation of the correct answer. These data suggest that LacApp is a useful tool to support students when learning the concept of gene regulation. We are interested in expanding the use of LacApp, so educators who are interested in using this tool in their undergraduate genetics course are encouraged to stop by for further discussion.

1868S **Incorporating whole genome sequencing and analysis into a high school teaching lab** Maitreya Dunham University of Washington

Evolution can be difficult to learn and teach. We have developed a yeast experimental evolution and genomics lab (“yEvo”) for high school students to learn about evolution by doing it. Briefly, students passage yeast cultures in increasing dosages of a stressor. Strains with enhanced resistance are then whole genome sequenced by the university lab partner, and the resulting mutations can be analyzed both by the high school students as part of their class and by the university team as part of our research on genome evolution. One as yet unsolved aspect of yEvo has been involving the students in genome sequencing and analysis. I’ll present some strategies and tools we are exploring to improve this aspect of our curriculum, primarily addressing data visualization. Sequencing and analysis are as yet still taking place at the university lab. However, new web-based tools for

viewing sequencing read "pileups" have made it more practical for students to look directly at their data. We have developed a short module to lead students through using the web app version of the Integrative Genomics Viewer. By viewing their sequencing data at the read level, students learn more about how modern sequencing works and can directly compare their evolved and ancestral strains. As a second goal, we wanted to facilitate students comparing data to their classmates, as well as with other yEvo classrooms. We developed a Shiny app mutation browser that allows students to explore mutations and easily click through to resources at SGD to learn about the genes of interest: https://yevo.org/mutation-browser/ We are deploying these new tools in classrooms during the 2023-24 school year. Having students participate in the read mapping and variant calling steps of the bioinformatics pipeline remains a goal, on which we would welcome ideas.

1869S **ORFans and proto-genes: Engaging students in bioinformatics through the study of yeast genes of unknown function** Jill B Keeney Biology, Juniata College

The model yeast *Saccharomyces cerevisiae* is arguably the most-studied eukaryotic model organism, yet nearly 10% of the annotated genes are of unknown function. Additionally, the yeast genome contains thousands of unannotated genes, possible proto-genes with potential for evolutionary functional selection over time. We have developed *in silico* laboratory modules that are directed at investigating genes of unknown function, as well as proto-genes yet to be annotated. Through the modules, students are guided through application of cellular and molecular concepts, including the central dogma, protein structure and localization, and gene evolution. The modules direct students in interpretation of algorithm results, leading to student-generated hypotheses of gene function. The study of yeast genes can be used to reinforce basic biological concepts and provide data for generating hypotheses. Modules are appropriate for introductory courses and can be adapted for upper-level courses.

1870S **Full-Immersion Research Experience (FIRE): a hybrid CURE + Research Internship** Cheryl Van Buskirk Biology, California State University Northridge

Course-based Undergraduate Research Experiences (CUREs) help build a foundation for a scientific careers and have the capacity to involve many students in novel research. These course-based experiences often encourage students to seek out research internships within a faculty member's lab, which in turn cultivates personal connections with a community of scientists. To facilitate this transition, California State University Northridge (CSUN) Biology has begun to implement an undergraduate research experience called FIRE (Full Immersion Research Experience) that leverages the strengths of both course-based and internship models and provides a supportive transition to independent research. With the support of NSF-IUSE funding, we are implementing and assessing the impact of this model on student interest in research-related careers and on the capacity of the CSUN Biology department to involve undergraduates in research.

1871V **Genetics Beyond Laboratories: Using Drosophila as a learning tool for biology in Nepalese Schools** Suvechhya Bastola[1], Yogesh Joshi[2], Vibha Acharya[3], Prajjwal Rajbhandari[1], M. Alba Abad[4] [1]RIBB, [2]Anatomy and Cell Biology, Wayne State University, [3]University of Pittsburgh, [4]Engage Nepal with Science

Drosophila has an enormous potential beyond research for biology teaching. It is a powerful modern teaching tool not only for classical genetics but for many curriculum-relevant areas of biology, providing unique access to empowering and inspiring classroom experiments. Our project *Games of Flies and Genes* encourages science educators in Nepal to work collaboratively with fly researchers and takes *Drosophila* to the classrooms to make genetics lessons more interactive and dynamic. This project brings teachers and students under a common umbrella of nature and science by shifting the traditional classroom-based pedagogy to a new experiential learning process through workshops on genetics and fly research combined with the use of microscopes to explore real flies and gamification tools. *Game of Flies and Genes* is run by *Engage Nepal with Science* (ENwS) a collaboration between the University of Edinburgh (UK) and the Research Institute for Bioscience and Biotechnology (RIBB, Nepal) that aims to spread the culture of engaging with science to empower, inspire and build confidence in STEM. This project is believed to add yet another milestone to our goal of creating a culture of empowerment in research in communities in Nepal.

1872V **Using course-based undergraduate research experiences (CUREs) to develop approaches for DNA barcoding ramshorn snails** Jessica Shinn-Thomas, Shakhrizoda Khazratkulova, Gladys Leitch, Katherine Hawley, Thomas McCarthy Biology, Utica University

Using course-based undergraduate research experiences (CUREs), we aim to develop approaches for species-level identification of ramshorn snails purchased from Ward's Science and Carolina Biological Supply Company that are used in our undergraduate student-faculty research programs. Neither company provides species-level classification, information about from where the snails are collected, if they are consistently collected from the same geographical location, or if they

are aquarium-reared. Students in our 300-level, Genetics laboratory course for science majors contribute to developing DNA barcoding approaches for the COI and 16S rRNA genes for identifying the species of ramshorn snails used in our undergraduate research programs. Students develop and reinforce their skills in molecular genetics including tissue isolation, DNA extraction, polymerase chain reaction (PCR), gel electrophoresis, and primer analysis. Some students independently continue the research after the course concludes to sequence and analyze the barcodes using bioinformatics. Our preliminary phylogenetic analyses show the ramshorn snails sold by both companies to be either *Planorbarius corneus* or *Planorbella trivolvis* and further confirmatory approaches are underway. Upon confirming our approaches, we will analyze local wild-caught snails and additional batches of Ramshorn Snails from both companies to determine consistency in species among separate purchases and between companies.

1873T **Unraveling the Pathophysiology of Jordan's Syndrome: A Proteogenomic Approach** Harris J Bolus[1], Kali Smolen[2], Jasmine Carter[3], Julian Halmai[3], Chenchen Li[4], Viktoriya Solodushko[4], Chelsie Carpenter[4], Justin Roberts[5], Mark Swingle[4], Kyle Fink[3], Arminja Kettenbach[6], Richard Honkanen[4] [1]College of Medicine, Whiddon College of Medicine at the University of South Alabama, [2]Geisel School of Medicine at Dartmouth, [3]UC Davis Neurology and Institute for Regenerative Cures, [4]Department of Biochemistry and Molecular Biology, Whiddon College of Medicine at the University of South Alabama, [5]Department of Pharmacology, Whiddon College of Medicine at the University of South Alabama, [6]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth

Jordan's Syndrome (JS) is a rare developmental disorder caused by *de novo* heterozygous dominant missense variants of the PPP2R5D gene. It presents with symptoms ranging from developmental delays to seizures and sensory challenges. The biological role of PPP2R5D and pathophysiology of JS are largely unknown, and no treatment has yet been found. We aim to elucidate the consequences of PPP2R5D mutations using cell culture models of the syndrome.

Using CRISPR-Cas9, cell lines recapitulating the E198K mutation were generated, including HEK-293 cells, and patient-derived induced pluripotent stem cells (iPSCs) and neural stem cells (NSCs) in which isogenic controls were generated by fixing the patient's single-nucleotide polymorphism. We combined nanopore-based RNA sequencing, traditional RNA sequencing, and TMT-based quantitative phosphoproteomics. We analyzed the output using the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Over-Representation Analysis (ORA). The significance threshold was $p < 0.05$ for RNAseq as well as HEK-293 phosphoproteomics. Due to greater sample size, an FDR threshold was calculated at $p < 0.0412$ and $0 < 0.016$ for iPSC and NSC phosphoproteomics, respectively, using the Benjamini-Hochberg procedure.

398 proteins were differentially expressed in all three proteomics datasets, including NAPRT (Nicotinate phosphoribosyltransferase), SLC3A2 (subunit of multiple large neutral amino acid transporters), and LDHA (L-lactate dehydrogenase A chain).

152 phosphopeptides were differentially phosphorylated in all three phosphoproteomics datasets, including KRT8, PPP1R12A (Protein phosphatase 1 regulatory subunit 12A), and AATF (Apoptosis antagonizing transcription factor, a possible HDAC1 inhibitor).

Overrepresentation analysis (ORA) of proteins, phosphorylation sites, and RNA transcripts with a significant change in abundance consistently revealed several KEGG pathways including spliceosome, RNA transport, regulation of actin cytoskeleton, proteoglycans in cancer, metabolic pathways, neurotrophin signaling, and insulin signaling.

Our findings suggest JS may involve the neuronal cytoskeleton, potentially affecting connectivity or morphogenesis. The prevalence of pathways and proteins associated with metabolism, cancer, and apoptosis hints at a broader metabolic disturbance. Additionally, the involvement of the spliceosome and RNA transport invite further research. Overall, our integrative proteogenomic approach provides insights into the multifaceted pathophysiology of JS and proposes novel candidate genes and proteins for future investigation.

1874T **Drosophila salivary gland transcription factors as novel targets for vector control** Bianca Palicha[1], Dorian Jackson[1], Deborah Andrew[1], Yuemei Dong[2] [1]Johns Hopkins University, [2]Molecular Microbiology & Immunology, Johns Hopkins University

The salivary gland (SG) secretions from mosquitoes and other vector species play essential roles in blood feeding, but also present a public health burden as the SGs serve as a gateway for transmission of a plethora of human pathogens. The genetic toolkit available for studying vector species is far less extensive than what is available in Drosophila melanogaster. More genetic reagents are necessary to gain further insights into vector biology. Interestingly, many of the key promoters currently utilized in vector research originated from Drosophila. As such, we are interested in leveraging our extensive understanding of SG development in flies to identify gene candidates with potential utility in vector-borne disease research. Sage is a basic helix-loop-helix (bHLH) transcription factor (TF) expressed exclusively in the SG of Drosophila melanogaster that regulates

expression of secreted cargo. During SG development, sage is activated at the onset of SG specification and persists for the duration of embryonic, larval, and adult life. Previous studies have also shown that sage is essential for cell viability during SG development, as null mutations resulted in SG apoptosis. Similar cell death is observed in silkworm moths in which genetic deletions of the sage ortholog resulted in the animals surviving with poorly developed silk glands. The Anopheles gambiae Sage ortholog AGAP013335 is also expressed in the SG at high levels across developmental stages. Additionally, a comparative analysis of Sage protein sequence and orthologs in other arthropods demonstrated a high degree of conservation in the bHLH binding domain across all primary mosquito vectors. Taken together, the tissue specificity coupled with its conserved developmental function across species make sage an ideal candidate for genetic studies in SG vector biology. Here, we describe our experimental approaches to characterize the function of AGAP013335 in mosquitoes as a potential target for vector control. Experiments are underway to test (1) whether Sage is functionally conserved in its capacity to bind DNA across insect species and (2) the effect of sage loss in A. gambiae mosquitoes.

1875T **Quantitative Analysis of Cytoplasmic Transfer between Human Retinal Organoids** McKaily Adams, Ying Liu, Mandeep Singh, Robert J Johnston Johns Hopkins

Degenerative photoreceptor diseases, such as age related macular degeneration and retinitis pigmentosa, are ever growing problems among older populations. As the retina lacks regenerative capacity, transplantation methods may restore functionality to degenerating photoreceptors. Stem cell-derived human retinal organoids have potential therapeutic applications for patients with degenerative diseases. When transplanted, organoid-derived donor photoreceptors transfer cytoplasm and mitochondria to host cells via tunneling nanotubes in a process called cytoplasmic transfer. Our goal is to use stable isotope labeling by amino acids in cell culture (SILAC) to quantitatively assess the transfer of proteins between donor and host cells. SILAC is a proteomics approach that allows 'heavy' isotopes of amino acids to be incorporated into proteins and identified by mass spectrometry. These studies will characterize the proteins that are transported during cytoplasmic transfer, suggesting how retinal organoid transplantation could transfer healthy material to degenerating host photoreceptors.

1876F **Nrf2/CncC and Hsf1 play a role in intestinal stem cell identity and gut homeostasis in *Drosophila*** Carlos L Quinones Sanchez[1], Heinrich Jasper[2], Jason Hackney[2], Airined Montes[3], Otto Morris[2], Alfredo Ghezzi[1], Imilce Rodriguez-Fernandez[1] [1]Biology, Universidad de Puerto Rico - Rio Piedras, [2]Genentech Inc, [3]Universidad de Puerto Rico - Rio Piedras

Adult somatic stem cells have a crucial role in maintaining the regenerative capacity of tissues throughout the lifespan of the organism. With age comes a reduction in tissue renewal and repair upon injury. Understanding the mechanisms that maintain stem cell function could potentially lead to the development of therapeutic strategies to mitigate age-related tissue degeneration and improve healthspan. Of particular interest is understanding how stem cells deal with different types of stressors such as oxidative stress and bacterial stress.

To study this, we use the fruit fly *Drosophila* intestinal stem cells as a model system. Intestinal stem cells have a crucial role in regenerating lost intestinal cells after bacterial infection, oxidative stress, or normal cell attrition. Thus, intestinal stem cells are always sensing the gut environment and respond to cues by activating or repressing proliferation.

By studying the function of two conserved stress-sensing transcription factors, namely Nrf2/CncC and HSF1 in intestinal stem cells (ISCs), we have uncovered an unprecedented role of these genes in stem cell identity. RNAseq experiments done in ISCs revealed that individually Nrf2/CncC and Hsf1 can repress a group of genes involved in proliferation. Unexpectedly, we found that both *Nrf2/CncC* and *Hsf1* repress the enteroendocrine (EE)-specification gene *asense*. Immunohistochemistry experiments show that when both *Nrf2/CncC* and *Hsf1* genes are knocked down in ISCs there is a significant accumulation of EE progenitors. This accumulation of EE progenitors led to an early onset of aging-like phenotypes such as microbial dysbiosis, leaky gut, and reduced survival. These findings could shed light on the mechanisms altered during aging that contribute to stem cell misdifferentiation and microbial dysbiosis.

1877F **Discovery of Drug Response-Associated Mutations in Mucinous Metastatic Appendiceal Cancer Using a Patient-Derived Tumor Organoid Platform** Daniel J. Gironda[1,2], Steven D. Forsythe[1,2,3,4], Cecilia R. Schaaf[1,2,4], Richard A. Erali[2,5], Ashok K. Pullikuth[1,2], Ming Leung[2,6], Shay Soker[2,4], Edward A. Levine[2,5,6], Konstantinos A. Votanopoulos[2,4,5,6], Lance D. Miller[1,2,6] [1]Department of Cancer Biology, Wake Forest School of Medicine, [2]Wake Forest Organoid Research Center, Wake Forest School of Medicine, [3]Endocrine Oncology Branch, National Cancer Institute, [4]Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, [5]Department of Surgery, Wake Forest School of Medicine, [6]The Atrium Health Wake Forest Baptist Comprehensive Cancer Center, Wake Forest School of Medicine

Metastatic appendiceal cancer (mAC) is an aggressive orphan disease that affects ~1/100,000 patients in the U.S. every year. Clinically, mAC presents as multiple tumor lesions colonizing spatially distinct sites throughout the peritoneal cavity, with no group yet to assess the molecular heterogeneity between distinct metastases in regard to drug responsiveness. With

IRB approval, we created patient-derived tumor organoids (PTOs) from 17 mAC patients treated at our institution to model the role of intra-patient tumor heterogeneity on drug responsiveness. We performed PTO drug screening with standard chemotherapies (FOLFOX, FOLFIRI) to enrich for chemo-sensitive or -resistant tumor cell subclones. PTOs derived from anatomically distinct locations within the same patient and treated with equimolar doses of FOLFOX or FOLFIRI showed, in some instances, differential drug responses as measured by ATP release. We selected 17 tumors from 6 patients that demonstrated robust chemo-sensitivity or -resistance between treatments for downstream whole exome sequencing (WES). PTO DNA was extracted from 51 total treated PTO groups (vehicle, FOLFOX, FOLFIRI) and 6 corresponding saliva samples (healthy controls), analyzed for integrity and quantity, then analyzed by WES using the Illumina NextSeq 6000 platform. Resulting data were processed and analyzed for mutation calls using GATK and DRAGEN pipelines. The variant allele frequency (VAF) was quantified and compared between vehicle-treated and FOLFOX or FOLFIRI-treated PTOs- an increase in VAF post-treatment represented possible chemo-resistance, with decrease in VAF representative of possible chemo-sensitivity. Recurrent mutations solely associated with chemo-resistant PTOs and present in ≥67% (n=4/6) of our mAC patients were associated with impaired G-coupled protein receptor function, dysregulated Wnt signaling, inhibition of DNA damage repair, and inhibition of intrinsic apoptosis. Alterations exclusive to chemo-sensitive PTOs and present in ≥50% (n=3/6) of mAC patients include negative regulators of fatty acid metabolism, inducers of epithelial to mesenchymal transition (EMT), and repressors of t-cell invasion. These preliminary data suggest that the underlying biology of chemo-sensitivity or -resistance in mAC may be predicted through the PTO-modeled identification of recurrent mutations across inter- and intra-patient lesions. Follow up analyses on a larger cohort of mAC PTOs will be needed to validate these findings.

1878S **Topoisomerase 3b enhances stability of maternal mRNAs that are essential for neurodevelopment of progeny** Seung Kyu Lee, Amy Qiang, Aaron Park, Dimitrios Tsitsipatis, Yutong Xue, Tianyi Zhang, Showkat Dar, Weiping Shen, Jennifer Martindale, Myriam Gorospe, Emmanouil Maragkakis, Weidong Wang National Institute on Aging/NIH

Topoisomerase 3 beta (TOP3B) is the only dual-activity topoisomerase in eukaryotes that can change topology for both DNA and RNA. Current evidence suggests that TOP3B can facilitate transcription on DNA, in addition to translation and turnover of mRNAs. Top3b forms a complex with Tudor domain containing 3 (TDRD3), which interacts with the Fragile X Mental Retardation Protein (FMRP) to regulate mRNA translation in neurons. In humans, the *Top3b* mutation has been linked to schizophrenia, autism, and cognitive impairment, whereas TOP3B inactivation in mice results in reduced lifespan and abnormal neurodevelopment. However, the detailed mechanism of how Top3b contributes to mental health and which genes are regulated by TOP3B at DNA and RNA levels remain unclear.

During the quiescent period of ovarian development, the oocyte remains transcriptionally inactive, yet there is a decline in mRNA translation. In *Drosophila*, a fully matured oocyte can be maintained in a quiescent state by restricting nutrition and mating, which provides an ideal environment for studying mRNA stability and translation in the absence of transcription. Here, we employed the quiescent oocyte model to investigate whether and how TOP3B-TDRD3 complex facilitates translation or degradation of maternal RNAs in oocytes. Furthermore, we explored the intergenerational effect of Top3b on maternal RNA maintenance by assessing progeny development.

Our results demonstrate that *Drosophila* embryos derived from the $Top3b^{-/-}$ or $Tdrd3^{-/-}$ oocytes under normal nutrition conditions develop normally. Conversely, embryos derived from oocytes with extended quiescent period exhibit infertility and abnormal neurodevelopment, characterized by severe defects in the ventral nerve cord (CNS) formation. These phenotypes resemble those observed in the embryos from $fmr1^{RNAi}$ flies, suggesting that the TOP3B-TDRD3 complex functions similarly to FMRP in promoting zygotic neurodevelopment. Our RNA-seq analysis revealed that a subset of mRNAs undergoes increased degradation in quiescent $Top3b^{-/-}$ oocytes, suggesting that these mRNAs rely on TOP3B for their stability. Furthermore, the TOP3B-regulated mRNAs overlap with those regulated by FMRP, implying a coordinated role for TOP3B and FMRP in mRNA degradation. Notably, the poly(A) tail length of the RNAs from the quiescent $Top3b^{-/-}$ oocytes exhibited a significant reduction compared to WT or $Top3b^{-/-}$ under normal condition, suggesting that Top3b promotes RNA stability by maintaining the poly(A) tail length. Together, our findings support a model that the TOP3B-TDRD3 complex works with FMRP to regulate maternal mRNA degradation in oocytes, and this function is critical for normal neurodevelopment in embryos.

1879S **A germline organoid model of *Taeniopygia guttata* (zebra finch) Primordial Germ Cell (PGC) migration *in vitro*** Bianca Brown, Ajuni Takkar, Naomi Greengold, Chennai Kaminski, Mary Collins, Michael Sanchez, Taraji Ellington, John Bracht American University

Primordial germ cells (PGCs) comprise an undifferentiated stem cell population that give rise to gametes. Set side during embryonic development, PGCs must migrate through the developing embryo to their destination, the gonads. The process has been studied in mammals and fish, but not in birds due to the shelled embryo. We developed an *in vitro* culture system for the growth of *Taeniopygia guttata* (zebra finch) organoids and observation of PGC migration into these structures. Our ultimate

objective is to study the function of the germline-restricted chromosomes (GRCs), an accessory chromosome only found in the germ line.

1880T **Tissue-Specific DAF-2 Degradation extends the lifespan and healthspan of *C. elegans* males** Rose S Al-Saadi, Hannah B Lewack, Patrick C Phillips Biology, University of Oregon

Aging is a universal phenomenon experienced by nearly all multicellular organisms, representing the primary risk factor for multimorbidity, as well as many chronic and neurodegenerative diseases. However, our understanding of the molecular mechanisms contributing to healthy aging remains limited. The nematode *Caenorhabditis elegans* has emerged as a pivotal model organism for uncovering genes and genetic pathways that regulate aging. This includes the identification of the remarkably long-lived *daf-2* mutants. The male mating behavior of *C. elegans* serves as an excellent model for the study of healthspan, given its robust and neurologically complex nature. Notably, in males, *daf-2* mutants not only exhibit exceptional longevity but also an extension of their reproductive healthspan, as measured by their mating success. However, due to the ubiquitous expression of *daf-2*, pinpointing the specific tissues where it regulates lifespan and reproductive healthspan has proven challenging. Recently, usage of the auxin-induced degron (AID) system has enabled researchers to determine that the targeted degradation of DAF-2 in the intestine plays a significant role in extending lifespan without influencing development or reproduction in hermaphrodites. Additionally, degradation of DAF-2 in other tissues, such as neurons, germline, and hypodermis, showed a slight but significant extension of hermaphrodite lifespan. However, the potential sex-specific effects of this tissue-specific degradation on lifespan and reproductive healthspan have remained unexplored. To address this gap, we used DAF-2 AID strains targeting specific tissues to investigate the effects of tissue-specific DAF-2 degradation on lifespan and reproductive healthspan in males. Our findings indicate that the degradation of DAF-2 in all somatic tissues, but not a specific tissue type, extends reproductive healthspan. This somatic degradation, akin to what occurs in hermaphrodites, also significantly extends male lifespan. This project contributes to a deeper understanding of how genetic sex influences the aging process and age-related decline.

1881T **Sex-specific larval viability effects of mutations in *Drosophila melanogaster*** Rob H Melde[1], JoHanna Abraham[2], Maryn R Ugolini[2], Madison P Castle[2], Molly M Fjalstad[2], Daneila M Blumstein[2], Nathaniel Sharp[2] [1]Integrative Biology, University of Wisconsin Madison, [2]Genetics, University of Wisconsin Madison

Mutations persist in all populations. Every generation some new mutations will be introduced, and other existing mutations will be removed (selection). Since new mutations tend to be deleterious, the fitness of a population is always lower than theoretically expected (mutation load). Selection acting on traits that directly impact reproductive success (sexual selection) is thought to reduce the mutation load experienced by females. Sex-specific selection (selection acting differently on males and females) often overlaps with cases of sexual selection, however since not every life history trait is directly involved in reproductive success there may exist examples of sex-specific selection that fall outside of the scope of sexual selection. One such life history trait that may be larval viability since during early development many genes have sex-biased expression. Here, in an effort to better understand the evolution and maintenance of sex, we investigate sex-specific viability selection. To do this, we test whether specific mutations or mutations more generally show evidence for a sex-bias in their fitness effects on larval viability. To test whether specific mutations experience sex-specific viability selection we measured relative viability of eight phenotypically dominant marker mutations in *Drosophila melanogaster*. We find that 2/8 mutations show sex-biased larval viability effects, where females experience greater reductions in viability than males. We also find that averaged across sexes, 5/8 mutations significantly decrease viability, and one significantly increases viability. We also find that a recessive allele shows a sex-biased viability effect, with a significant decrease in males and no change in females. To test whether mutations more generally show sex-specific viability effects, we utilized mutagenesis to measure sex-specific viability of 33 genotypes containing many randomly induced mutations, relative to an unmutagenized strain. We find that mutations, on average, show sexually concordant viability effects, where they tend to decrease viability similarly in both males and females. Our results indicate that while viability selection on average is sexually concordant, certain specific mutations show sex-biased viability effects (in both directions). Here we provide novel evidence that sex-specific selection can act prior to adulthood by providing evidence of sex-biased fitness effects in multiple commonly used *Drosophila melanogaster* marker mutations. Future studies of sex differences in selection should not ignore the possibility of sex-specific selection prior to adulthood.

1882T **Tdrd5l promotes male identity in germline stem cells** Caitlin Pozmanter, Mark Van Doren Biology, Johns Hopkins University

Germline sex determination is regulated by a combination of signals from the somatic gonad, and germline autonomous sexual identity regulated by the RNA-binding protein Sex lethal (Sxl). Previously our lab identified *Tudor domain containing protein5-like (Tdrd5l)* as being important for male identity in the germline. Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Tdrd5l is

expressed in the developing germ cells and GSCs in males but is repressed in female GSCs at least in part due to regulation by Sxl. Currently we are working to understand how Tdrd5l promotes male germline identity.

Tdrd5l mutant adult testes exhibit dramatic germline loss which is often seen in mutants for sex determination factors where the sex of the germline does not match the sex of the surrounding soma. One important regulator of germline sex determination is the JAK/STAT pathway, which promotes male germline identity but is repressed in female germ cells downstream of *Sxl*. Interestingly, *Tdrd5l* is also important for regulating germline JAK/STAT activity. In *Tdrd5l* mutants, male GSCs showed a reduction of Stat staining indicating a loss of JAK/STAT signaling. Another important signaling pathway active in the GSC niche is the BMP pathway. Our work shows that BMP signaling (pMAD) in GSCs is sexually dimorphic and is substantially higher in female GSCs compared to male GSCs. Loss of *Tdrd5l* in the male germline results in increased pMAD expression in male GSCs suggesting possible feminization of these cells. Further, expression of Tdrd5l in the female germline led to a decrease in GSC pMAD levels comparable to male GSC pMAD levels. Thus, these data support a model where *Sxl* and *Tdrd5l* act autonomously in the germline to regulate key signals from the somatic gonad acting through both the JAK/STAT and BMP pathways to control germline sexual identity. Tdrd5l localizes to a perinuclear body similar to, but distinct from, the nuage. Current work focuses on understanding the composition and function of the "Tdrd5l body" and how it influences signaling from the somatic gonad to the germline.

1883T **Female specific increased Insulin/insulin-like growth factor signaling pathway promotes increased body fat in *Drosophila* females than males** Puja Biswas, Colin Miller, Elizabeth Rideout Cellular and Physiological Sciences, University of British Columbia

Female flies store more body fat than males. While growing evidence suggests catabolic factors contribute to the sex difference in body fat by restricting male fat storage, less is known about the metabolic pathways that promote fat storage in females. Given its known role as a positive regulator of fat storage, we performed a detailed investigation of the insulin/insulin-like growth factor signaling pathway (IIS) in male and female adult flies. We found that mRNA levels of *Drosophila insulin-like peptides* (*dilps*) were higher in females, and that females had higher peripheral insulin sensitivity. IIS activity in the abdominal fat body was also higher in females than in males. To test whether increased IIS activity contributes to increased fat storage in female flies, we genetically ablated the insulin-producing cells (IPCs) in adult flies and measured body fat. IPC ablation significantly reduced body fat in females but not in males. In line with previous findings, females with ablated IPCs had decreased offspring production. Together, our data suggest that IIS activity is higher in females, and that this elevated activity contributes to the sex difference in body fat by promoting fat accumulation in females. This reveals previously unrecognized sex differences in IIS regulation, and identifies IIS as an additional metabolic pathway that contributes to the sex difference in body fat.

1884T **Y chromosome toxicity does not contribute to sex-specific differences in longevity** Renald Delanoue, Charlene Clot, Chloe Leray, Thomas Pihl, Bruno Hudry Institut de Biologie Valrose, Université Côte d›Azur, CNRS, Inserm

Men and women are phenotypically different. Height, weight, disease prevalence or life expectancy are sex-biased, but the origin of these differences is still not well understood. Some studies suggest that differences in longevity are due to the intrinsic presence of the sex chromosomes, and in particular due to the Y chromosome structure. A correlation between Y chromosome number and longevity was observed, since life expectancy is reduced in individuals with supernumerary Y chromosomes. In the animal kingdom, heterogametic sexes (the males in mammals or some insects, the females in birds or reptiles) have shorter lifespan, and this led to the hypothesis of a "toxic effect" of the Y (or W) chromosome. These chromosomes, mostly composed of simple repeated sequences and transposable elements, are transcriptionally silenced in large heterochromatic domains. In *Drosophila*, we measured that the Y chromosome represents 13% of the male genome and these large heterochromatin structures are absent in females. During aging, heterochromatin marks are lost, transposons become derepressed and transpose, potentially generating deleterious mutations and faster aging. It suggests that the Y chromosome-associated toxicity might impact the male physiology according to its size. This led us to test whether Y chromosome size has any consequences on sex-biased phenotypes and longevity. To this aim, we developed an innovative CRISPR/Cas9 strategy in *Drosophila* using gRNAs to target specific repeated sequences of the Y chromosome. With this method, we generated a library of Y chromosomes with different sizes or complete loss. Using this library in combination with a reporter gene, we demonstrated that the size of the Y chromosome can affect heterochromatin maintenance in the whole genome. However, we measured how Y chromosome presence or size modifies *Drosophila* lifespan and to our surprise, we revealed that the amount of Y chromosome heterochromatin does not change fly lifespan. The hypothesis of the Y chromosome-associated toxicity cannot explain sex differences in lifespan. Instead, we could show that sex-biased life expectancy is essentially controlled by the sex determination pathway itself, through the female-sex determinant, *transformer*.

1885T **Investigating the molecular basis of sex-biased brain aging in *Drosophila melanogaster*** Nathan Nigrin, Gunjan Singh, Kaitlyn Cortez, Kate O'Connor-Giles, Erica Larschan Brown University

Sex-biased aging differences, despite being widely observed in diverse species, are not well understood. Even further, there is a growing desire to understand the basis of the significantly sex-biased progression of aging and neurodegeneration in humans. To address this problem, we are investigating dosage compensation, the process in which X chromosome-linked genes are upregulated in XY males to equalize their expression to XX females, for a potential role in the aging brain. We have previously shown that dosage compensation is highly dysregulated in the aging male Drosophila brain and are thus interested in examining whether this process is linked to sex differences in aging phenotypes.

Methodologically, we investigate how the downregulation of MSL2, MLE, and CLAMP, required components of the dosage compensation complex (DCC) in Drosophila, may decrease lifespan in males, where the complex is normally active. We use a modified UAS-Gal4 system known as the geneswitch system to temporally control RNAi-mediated DCC component knockdown in adults, thus inhibiting dosage compensation. We then study these populations through survivorship assays, molecular techniques such as qPCR and western blots, and computational methods, including RNA-seq and ATAC-seq to assess sex-specific differences in aging brains. In a parallel approach, we are using the CRY2-blue light system to temporally inhibit MLE function, followed by assessment of differences in transcription, translation, and chromatin accessibility. Early results have indeed shown shortened lifespan in MLE-inhibited individuals; an exciting result as we move forward with our more mechanistic experiments.

Separately, we are measuring the role of histone modifications to Drosophila chromatin, which have been used to approximate age as an epigenetic "clock." H4K16ac chromatin marks are deposited by the DCC to modulate chromatin accessibility of X chromosome genes, and thus are an effective way of studying dosage compensation's impact on aging flies. We use both bulk histone proteomic methods and the genomic technique of CUT & RUN to measure the presence and localization of these histone acetylation marks on chromatin to elucidate any differences between male and female brains throughout normal aging. Overall, these experiments will bring us closer to an understanding of sex-based differences in aging, specifically in the brain.

1886T **GA binding transcription factor CLAMP regulates the dynamics of splicing condensates in *Drosophila*** Smriti Vaidyanathan, Mukulika Ray, Erica Larschan Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Many RNA binding proteins (RBPs), part of the spliceosome complex, form nuclear condensates through phase separation. However, how spliceosome condensates are regulated and their impact on splicing function remains unclear. Furthermore, while extensive sex-specific splicing is reported in all organisms, the influence of the dynamics of spliceosome condensates on it remains unexplored. In this present study, we investigated the dynamics of Hrp38 (homologous to the human HnRNPA2B1 family protein), a splicing component involved in many human neurodegenerative disorders, in *Drosophila* larval tissues. Additionally, for the first time, we explored the dynamics of another conserved component of the spliceosome complex, RNA helicase A (MLE), and compared the dynamics of MLE and Hrp38 in sex-specific cells. An earlier study identified CLAMP (chromatin-linked adaptor for male-specific lethal proteins) as a transcription factor integral to *Drosophila* sex-specific splicing. CLAMP regulates splicing function via interactions with RBPs in spliceosomes, and our computational analysis revealed that CLAMP shares binding sites with many splicing-involved RBPs. CLAMP also undergoes phase seperation due to the presence of a PRLD domain. Therefore, we hypothesize that CLAMP sex-specifically regulates the function, behavior, and dynamics of RBP nuclear condensates. To investigate the role of CLAMP in the dynamics of MLE and Hrp38, we utilized *Drosophila* CLAMP mutant and CLAMP RNAi backgrounds and live-cell imaging techniques. We employed the software package TrackIt and developed a robust pipeline for image processing, particle detection, tracking, and data analysis to perform a quantitative analysis of the behavior of MLE and Hrp38 in nuclear condensates under different conditions. Our preliminary results suggest distinct differences in the dynamics of MLE and Hrp38 in nuclear condensates between female and male CLAMP mutant and wild-type samples. In conclusion, by combining live-cell imaging and computational analysis, we gain a deeper understanding of the dynamics of RBPs and their significance in gene regulation, offering potential avenues for future research in splicing mechanisms. Sex-based differentiation remains an area of ongoing research with significant implications for healthcare. Enhancing our insight into the regulation of sex-based differences in brain function and disease susceptibility has the potential to revolutionize healthcare across diverse populations.

1887T **Germline novelty through recurrent copy-number, protein, and regulatory evolution of the synaptonemal complex** Kevin Wei University of British Columbia

The synaptonemal complex (SC) is a protein-rich structure necessary to tether homologous chromosomes for meiotic recombination and faithful segregation. Despite being found in most major eukaryotic taxa implying a deep evolutionary origin, components of the complex can exhibit unusually high rates of sequence evolution, particularly in *Drosophila* where orthologs of several components could not be identified outside of the genus. To understand the cause of this paradoxical lack of conservation, we examine the evolutionary history of the SC in Drosophila, taking a comparative phylogenomic approach with

high species density to circumvent obscured homology due to rapid sequence evolution. We find that in addition to elevated rates of coding evolution due to recurrent and widespread positive selection, components of the SC, in particular the central element *cona* and transverse filament *c*(*3*)*G* have diversified through tandem and retro-duplications, repeatedly generating paralogs with novel germline functions. Strikingly, independent *c*(*3*)*G* duplicates under positive selection in separate lineages both evolved to have high testes expression and similar structural changes to the proteins, suggesting molecular convergence of novel function. In other instances of germline novelty, two *cona* derived paralogs were independently incorporated into testes- expressed lncRNA. Surprisingly, the expression of SC genes in the germline is exceedingly prone to change suggesting recurrent regulatory evolution which, in many species, resulted in high testes expression even though *Drosophila* males are achiasmic. Overall, our comprehensive study recapitulates the adaptive sequence evolution of several components of the SC, and further uncovers that the lack of conservation not only extends to other modalities including copy number, genomic locale, and germline regulation, it may also underlie repeated germline novelties especially in the testes. Given the unexpected and frequently elevated testes expression in a large number of species and the ancestor, we speculate that the function of SC genes in the male germline, while still poorly understood, may be a prime target of constant evolutionary pressures driving repeated adaptations and innovations.

1888T **The role of X chromosome dosage compensation during development** Océane Tournière[1], Seth Cheetham[2], Andrea Brand[3], Irene Miguel-Aliaga[4], Bruno Hudry[1] [1]Institut de biologie Valrose, Université Côte d'Azur, [2]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, [3]Gurdon Institute, University of Cambridge, [4]The Francis Crick Institute

Sex chromosomes control sex determination in most organisms. For example, in mammals and Drosophila, females with two X chromosomes have twice the number of X-linked genes compared to males, who only have one dose. In flies, the activation of a dosage compensation complex in males ensures that the level of most of these genes' products is the same in both sexes. This process is critical for male survival because a lack of any protein of this complex results in male-specific lethality. Despite extensive characterisation of the molecular mechanisms driving dosage compensation, its physiological contexts and the reasons behind lethality remain unclear. The exact biological functions of dosage compensation, the developmental processes and X-linked genes sensitive to abnormal X dosage have not been isolated so far.

To identify the developmental stages and organs that require dosage compensation, we conducted tissue-specific experiments. Our screening process, involving 250 cell-specific lines, revealed that dosage compensation is necessary for male survival in one specific tissue - the tracheal cells at pupal stage. Using RNA-DamID, we mapped and compared the binding profiles of the dosage compensation complex in tracheal cells with those in organs where the complex is dispensable. This approach identified of a small set of X-linked genes compensated only in tracheal cells. We are currently restoring the dose of these candidates, one by one, to rescue tracheal-specific dosage compensation silencing and identify developmental processes behind dosage compensation lethality.

We identified the genes and cells and are studying the processes causing male lethality due to X chromosome imbalance. Our results provide insights into the causative mechanisms responsible for dosage compensation of the sex chromosomes.

1889T **Investigating the Origin of Prostate Macrophages through Genetic Lineage Tracing** Peri Wivell, Andrew Pletcher, Maho Shibata The George Washington University

Prostate tissues contain heterogeneous populations of immune cells. Published single-cell RNA sequencing data from mouse and human prostates show that macrophages constitute a large portion of immune cells in the prostate. Under disease conditions, different subsets of macrophages can either promote or resolve inflammation, causing tissue damage or aiding in tissue repair. Previously, it was believed that tissue-resident macrophages were maintained and repopulated by blood-circulating monocytes derived from progenitors in adult bone marrow (BM). More recent studies have revealed that several tissue-resident macrophage populations arise from embryonic precursors in the yolk sac (YS) or fetal liver (FL) prior to birth and continue to maintain themselves throughout adulthood independent from BM-derived precursors. We hypothesized that distinct populations of macrophages in the mouse prostate arise from embryonic origins and contribute to the heterogeneity of macrophages in the prostate. To identify embryonic origin macrophages in the mouse prostate, we genetically labeled macrophages using a yellow fluorescent protein (YFP) reporter mice. Prostate tissues containing lineage-labeled cells were then collected and characterized during different stages of postnatal organogenesis: prior to, during, and after puberty. Through immunohistochemical and immunofluorescence staining for YFP, macrophage marker F4/80 and proliferation marker Ki-67, we show that macrophages of embryonic origin exist in the prostate. Further, these cells proliferate locally within the prostate and remain in the tissue after puberty. Our findings suggest that tissue-specific macrophages of embryonic origin may function to regulate prostate organogenesis. Understanding the function of tissue-resident macrophages during normal prostate organogenesis and applying this knowledge to prostatic diseases such as benign prostatic hyperplasia will further

expand our understanding of immune cell dysregulation.

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1890T **Why is there a *maleless* homologue in one of the homomorphic sex chromosomes of the *Aedes aegypti* mosquito?** Christen Hughes[1,2], Chujia Chen[3,4], Mark Potters[1,4], Zhijian Tu[3,4] [1]Biochemistry, Virginia Tech, [2]Fralin Life Sciences Institute, [3]Genetics Bioinformatics and Computational Biology Program, Virginia Tech, [4]Fralin Life Sciences Institute, Virginia Tech

The *Aedes aegypti* mosquito is a major vector for dengue, chikungunya, and Zika viruses. In contrast to *Drosophila melanogaster,*which has well-differentiated heteromorphic X and Y chromosomes, only small regions differ between the homomorphic sex-determining chromosomes in *A. aegypti,* and these regions are known as the M and m loci. There has been a concerted effort investigating the M locus which contains two genes *Nix* and *myo-sex*. *Nix* is the male determining factor and shown to masculinize females, and *myo-sex* is required for flight in males. Conversely, the m locus has not been extensively studied and the current m-locus assembly has 44 gaps within this 1.2Mb region. Our lab has generated a more continuous haplotype-resolved assembly. Within the newly assembled m locus, we identified a sequence that has a 65% amino acid identity to an autosomal orthologue of the *maleless* gene in *D. melanogaster* which encodes for an RNA helicase that is involved in dosage compensation. While dosage compensation is not expected in *A. aegypti*, the presence of an m locus *maleless* homologue (mMH) is of high interest. However, it is not clear whether mMH is a functional paralog of *maleless* or a pseudogene. We have preliminary evidence that mMH is present only on the m chromosome and it is transcribed in *A. aegypti* early embryos. We are determining the transcriptional profile of mMH and surveying its distribution in various strains of *A. aegypti*. We will investigate the function of mMH by knocking out mMH. This research will inform on sex determination within *A. aegypti*, which can be applied to sex ratio distortion efforts that decrease the ratio of biting females.

1891T **Identifying the sex determination gene *doublesex* in *Stiphra sp*. by degenerate PCR** Bailey Baratka[1], Stephen Madigosky[2], Alexis Nagengast[1] [1]Biochemistry, Widener University, [2]Biology, Widener University

Sex determination (SD) is the process that results in differences between males and females of a species initiated by chromosomal differences. Sex determination (SD) varies between species, but the similarities between the pathways can be examined using an evolutionary perspective. Extreme differences between male and females is known as sexual dimorphism and often found in insects. One of the best characterized SD pathways is in the fruit fly *Drosophila* and several gene products have been identified, such as *doublesex (dsx)*. The gene *dsx* has been found in multiple species of insects that are more distant in the evolutionary tree from fruit flies, such as beetles (Coleoptera). This is consistent with the hypothesis that the SD pathway evolved from the end of the pathway to the beginning. Jumping sticks (*Stiphra*) are more distant from fruit flies in the phylogenetic tree of insects than beetles. Therefore, we hypothesize that *dsx* will have sex-specific expression that results in the sexual dimorphism of jumping sticks. Previous work amplified genomic sequence with *Cyclommatus metallifer finae* (stag beetle) degenerate PCR primers and was concluded to not be *doublesex* sequence and showed that the DNA sample was contaminated. Therefore, new DNA was isolated from a female jumping stick and work is ongoing with newly designed primers to clone PCR products into *E. coli* before sequencing. This should allow for the isolation of dsx in an attempt to identify the SD pathway in jumping sticks.

1892F **Histone methyltransferases differentially regulate transcription and chromosome structure in oogenesis and spermatogenesis** Carolyn Remsburg[1], Lacole Fung[1], Lauren Salvitti[2], Aimee Jaramillo-Lambert[1] [1]Biological Sciences, University of Delaware, [2]Charles River Laboratory

Meiosis is the specialized cell division that results in the formation of haploid gametes from a diploid cell. During this process, duplicated chromosomes must undergo significant condensation and compaction before segregating into daughter cells. As defects in this process can result in aneuploidy, a leading cause of infertility and miscarriages, meiosis must be tightly regulated. Condensation and compaction are regulated by condensins, cohesins, and specific post-translational histone modifications, and there are sex-specific differences in this process. In *C. elegans,* bivalents at diakinesis (the last stage of prophase I) are significantly longer in oocytes than in sperm. Our lab identified two histone methyltransferases that differentially impact bivalent length in the male versus female germline. MET-2 catalyzes the dimethylation, and SET-25 catalyzes the trimethylation of histone H3 lysine 9 (H3K9me2 and H3K9me3). In *met-2* null worms, bivalent length during spermatogenesis is significantly shorter than in wild-type (WT); while in *met-2; set-25* worms bivalent length during spermatogenesis is significantly longer than in WT. Bivalent length during oogenesis in *met-2* worms is not significantly different than in WT worms. *met-2* and *met-2; set-25* worms have reduced brood sizes and embryonic viability compared to

WT, indicating that chromosome compaction contributes to gamete formation and quality. We also measured bivalent length in *him-17* mutant worms, as they have reduced H3K9me2 in both hermaphrodite and male gonads. Similar to *met-2*, bivalent length is shorter during spermatogenesis than in WT worms. However, shorter bivalents are also observed in oogenesis in *him-17* compared to WT. Immunolabeling showed that MET-2 localizes to germline nuclei through diplotene in hermaphrodites but remains localized through diakinesis in males. This suggests that MET-2 may function later during spermatogenesis than oogenesis. As H3K9 methylation is associated with transcriptional repression, we used immunolabeling for active RNA polymerase II (RNA pol II) to assay for global transcription. Results indicate germline nuclei in *met -2* males have more active RNA pol II during diplotene compared to WT indicating that changes in transcription may contribute to the reduction in brood size and embryonic viability. By dissecting the sex-specific regulation of chromosome structure and transcription during meiosis, we will elucidate key mechanisms of this essential process.

1893F **The impact of inter-organ communication on ovulation following traumatic injury in *Drosophila*** Cameron Dixon, Ethan Anderson, Kim McCall Biology, Boston University

Traumatic injuries (TIs) from intimate partner violence, vehicular collisions, high-impact sports, and even mundane activities can be fatal. However, survivors of TIs can have residual pathophysiological disturbances post-injury that lead to life altering conditions, including neurodegenerative diseases, mental illness, and metabolic disorders. Reproductive issues are a known consequence of TI especially in women, however this has remained poorly understood. These issues with reproduction are seen through menstrual cycle dysregulation, lower libido, decreased fertility, and increased rates of miscarriages. These consequences are not only localized at the time of the injury but can persist through the remainder of the individual's life. *Drosophila melanogaster* have recently emerged as a stellar model of TI due to relatively short lifespans, conservation of molecular/cellular cascades following injury response, and avoid many of the ethical dilemmas of using mammalian models. Reproductive consequences had not previously been tested using the *Drosophila* model and we have found that reproductive consequences are conserved. These reproductive consequences come in the form of decreased egg laying behavior and the retention of mature egg chambers, mimicking issues in ovulation. Investigation into the genetic and cellular mechanisms of these reproductive responses is underway. We hypothesize that hormonal disruption following TI leads to this change in egg laying and retention. Our recent data suggest that insulin may be the culprit for these reproductive issues. Insulin signaling and function is highly conserved in *Drosophila* and it has been shown that altered insulin production affects ovary health. Interestingly, patients who have undergone TI have higher rates of hyperglycemia and diabetes, implicating insulin disruption in humans following TI. These observations suggest that insulin is a prime candidate to be investigated further for its role in reproductive consequences following TI. Experiments are currently underway to tease apart which tissues are important for insulin expression for reproductive health and also how TI affects the expression profiles of other hormonal signaling pathways.

1894F **Using long-read sequencing data to analyze sex-based differences in alternative splicing in different species of *Drosophila*** Kinfe Bankole[1], Adalena V Nanni[2], Netanya Keil[1], Olga Barmina[3], Alison Morse[1], Ana Conesa[4], Lauren M McIntyre[1] [1]Molecular Genetics and Microbiology, University of Florida, [2]UPMC Genome Center, [3]University of California, Davis, [4]Institute for Integrative Systems Biology, Spanish National Research Council

Alternative splicing is a fundamental regulatory process. Alternative splicing results in structural differences between transcripts including: alternate donor/acceptor sites, alternate exon cassettes, intron retention, alterative transcription start sites and alternative polyadenylation sites. These different transcripts isoforms are now directly measurable due to advancements in long read sequencing technology. However, the variation in the observed transcripts from this technology poses a challenge in thinking about how best to quantify isoform expression from long reads. For protein coding genes, the structural differences between transcripts can be organized by the potential target protein. We develop an analytical approach that begins with raw long read data, then maps these reads to a reference annotation. Any transcripts with the same junctions are identified. We identify a set of possible similar proteins based on grouping transcripts that contain overlapping exons. We then determine whether the long-read transcripts are likely to make a protein in order to quantify isoform expression. Using this approach, we quantify gene and isoform expression in male and female head tissue for three species of *Drosophila*: *melanogaster*, *simulans*, and *serrata*, and identify conserved and divergent sex-specific isoforms.

1895F **Understanding the role of exosomes in competitive male reproductive success** Yoko A Takashima, Nora Brown, Alexandra Hoff, Ben R Hopkins, Andrew G Clark, Mariana F Wolfner Molecular Biology and Genetics, Cornell University

Male x female interactions at the behavioral, physiological, and molecular levels can profoundly influence the outcomes of mating encounters. In *Drosophila*, males transfer seminal fluid proteins (Sfps) and other factors to females, inducing physiological and behavioral changes known as the post-mating response (PMR). Disruption of Sfp production by the male impacts the female PMR in a way that reduces male reproductive success. Most factors responsible for the PMR are produced in the male accessory gland, a secretory epithelial tissue composed of two morphological cell types - main and secondary

cells. While only comprising 4% of the secretory cell tissue, secondary cells are essential for a male's reproductive success. In addition to synthesizing specific Sfps, these cells secrete exosomes, minute lipid-bound vesicles that encapsulate and transfer proteins and RNAs to the female and to sperm. We hypothesize that variation in exosome transfer among different genetic lines will impact sperm competition outcomes. We are exploring the natural variation of exosome production by immunofluorescent staining and confocal microscopy to quantify exosome abundances within wild-derived lines of flies such as the *Drosophila* Genetics Reference Panel (DGRP). With these techniques, we will utilize a genome-wide association (GWAS) approach to identify previously unknown genetic variants that impact exosome biology, with a particular focus on sperm competition outcomes. To extend this, we are developing a protocol to purify, quantify, and characterize exosomes and their cargo to dissect how exosome contents impact sperm competition outcomes.

1896F **The Role of Octopamine in *Drosophila* Exercise Response** Annie Backlund, Laura K Reed Biological Sciences, University of Alabama

Thirty four percent of people in the United States have metabolic syndrome: a cluster of symptoms such as obesity, high blood pressure, and insulin resistance. Metabolic syndrome greatly increases the chance of developing type-2 diabetes and heart disease, which are leading causes of death in the United States. Treatments for metabolic syndrome often include lifestyle changes such as increasing exercise; however, little is known about why males often exhibit greater adaptations to exercise than females in both humans and *Drosophila*. The Power Tower is a device that exercises flies by taking advantage of their negative geotaxis, which is a directional movement against gravity. Previous *Drosophila* studies have found octopamine to be essential for flies' adaptational response to chronic exercise training. Octopamine is a catecholamine found in invertebrates that is structurally and functionally similar to norepinephrine in humans. Norepinephrine acts as a hormone and neurotransmitter in the sympathetic "fight or flight" response to stress such as exercise. In humans, the nervous system releases epinephrine and norepinephrine in order to mobilize fuel substrates, increase blood pressure, and increase heart rate in order to meet the physiological demands of exercise. Several weeks of exercise training also increases an individual's capacity to secrete epinephrine/norepinephrine to more robustly respond to a stressful event. Previous studies have found that male flies exhibit a greater response to exercise than females due to differential expression of octopaminergic neurons. However, little work has been done that compares the sexes using multiple genetic lines. We will evaluate how males and females of several wild-derived genetic lines of *Drosophila* adapt to exercise and elucidate the role of octopamine in these responses. We will train several DGRP lines on the Power Tower and compare male and female climbing performance and time to fatigue before and after a two week long training program. Also, we will feed unexercised flies octopamine to better understand how this compound contributes to sex differences in response to exercise. We hypothesize that octopamine-fed flies will exhibit exercise adaptations even though they did not undergo the training program, and octopamine feeding will rescue females' exercise intolerance.

1897F **Quantifying the impact of biological sex and genetic background on the response to nickel toxicity using *Drosophila melanogaster*** Allie Hutchings, Jesse Petahtegoose, Alexis Okengwu, Claudia Taylor, Thomas Merritt Laurentian University

Females and males are biologically different. However, the sexual dimorphic effects we find in one genetic background, or genotype, may contrast in another. Taken together, both biological sex and genetic background work to provide broad scale conclusions of biological processes. In this study, we use the *Drosophila melanogaster* model system to compare both sexes of isogenic, genetically identical flies and wild caught, genetically diverse populations of flies to study the effects of nickel (Ni) toxicity on an organism-wide level. We use multiple isogenic lines to control for different genetic backgrounds and to compare sex differences. In contrast, we use wild-derived lines of flies to obtain generalized sex specific responses to stress that is more indicative of a natural population. We look at broad scale responses through mortality assays for both Ni toxicity and starvation, as well as more specific responses through enzyme activity, total lipid, and feeding assays post Ni exposure. In whole, we find that isogenic lines are more sensitive to stress than wild lines. Across the isogenic lines, our results clearly show that genetic background effects are significant but are more substantial across females than males. Furthermore, the sexual dimorphic effects are not consistent across genetic backgrounds. In fact, we find that in two isogenic backgrounds there are virtually no sex differences, whereas another line has an ~60% difference between males and females. Wild lines show less differences across lines but consistently found an ~55% difference between the sexes. Future work will utilize multiple metal contaminants to broaden our understanding of the metal interaction network. Overall, our results show that the magnitude of stress differs across both sex and genetic background, highlighting the importance of considering both in experimental design.

1898F **An Odorant-Binding Protein involved in courtship behaviour of *Drosophila melanogaster*** Enisa Aruçi[1], Stéphane Fraichard[2], Isabelle Chauvel[2], Lucie Moitrier[3], Jean-François Ferveur[2], Loïc Briand[3] [1]Cornell University, [2]University of Burgundy, [3]INRAe

Odorant binding proteins (OBPs) constitute a family of soluble carrier proteins mostly found in the sensillar lymph of

chemosensory hairs. They are not only involved in the chemoreception but also in several other function (Rihani *et al.*, 2021). In our project, we used *Drosophila melanogaster* as a model organism, to find a link between the presence of bacteria, the expression of OBPs and behavioural performance of flies. More particularly, we investigated the expression of OBP56d, present in the adult chemosensory system and in the gut, and we measured the influence on reproductive-related behaviours. We used both *in vitro* and *in vivo* approaches to characterize several aspects pertaining to OBP expression in adult gustatory appendages, gut and reproductive organs.

To carry out this project, we analysed by quantitative reverse transcription-polymerase chain reaction (RTq-PCR) the expression profiles of this OBP in different body parts (thorax, head, and intestine) in males and females. These flies were exposed to fasting periods of various durations. Beside its expression in distinct regions of the gut, and in the proboscis, it was also detected in male reproductive organs suggesting that it could be involved both in nutrition and reproduction. The effect of OBP56d was studied on female and male courtship behaviours by using OBP56d mutant and control drosophila w^{118}. Our data showed a decrease of mating by 33% between mutant drosophila (55%) and w^{118} (88%). We observed mating between mutant female/male and w^{118} female/male. The decrease of mating between mutant female and w^{118} male was by 47%. On the other hand, mutant male and w^{118} female had nearly the same percentage of mating like the controls one (85%). Also, we used competitive fluorescence binding assay to determine its binding properties to a broad panel of potential ligands.

Based on this data, we can conclude that the absence of OBP56d has an impact on female behaviour during mating. As we know, the recognition between members or cells of the opposite sex is an important ingredient in the success of mating and our findings indicate that OBP56d may be implicated in perception of chemical signal during mating.

1899F **Investigating how germline sexual identity controls sex-specific gene expression** Harrison Curnutte, Mark Van Doren Biology, Johns Hopkins University

In nature, animal species often exhibit sexual dimorphism, or differences in morphology and behavior between sexes. An important difference between the sexes are the gonads, the testis and ovary, which produce the sperm and egg necessary for sexual reproduction. In order to develop proper gametes, both the germ cells and the somatic cells of the gonad must decide their sexual identity. The RNA binding protein Sex lethal (Sxl) has been shown to be a master regulator of somatic sex determination and is expressed based on the presence of two X chromosomes in the female as opposed to one in the male. Sxl is also important in the germline, where it is both necessary and sufficient for female germline identity. However, autonomous sex determination downstream of Sxl is not well characterized in the germline. Interestingly, germline sex determination has also been shown to be regulated in a non-autonomous manner via somatic signals. How sex determination in the germline is regulated by a combination of autonomous cues, downstream of *Sxl*, and non-autonomous cues, based on somatic cell signaling, is unknown and of great interest to the field.

A key aspect of sex determination is the control of sex-specific gene expression, about which little is known in the germline. We will use expression of *Tdrd5l* (Tudor domain-containing protein 5-like) as a model for understanding sex-specific gene expression. Tdrd5l is expressed in a male-specific manner in the early germline and is important for male germline sexual identity. *Tdrd5l* RNA and protein are initially expressed in the embryonic germline of both sexes, but subsequently become male-specific by an unknown mechanism. At the third larval instar (L3) stage, Tdrd5l protein and RNA are both absent from female germ cells while they remain highly expressed in males, suggesting possible transcriptional regulation. Surprisingly, we have found that sex-specific expression of *Tdrd5l* RNA in developing germ cells is independent of *Sxl*. Additionally, we have found that a male soma is sufficient to drive expression of *Tdrd5l* RNA in female germ cells. However, *Tdrd5l* is regulated independently of JAK/STAT signaling, which is a key male-specific signal regulating germline gene expression. Altogether, our study of *Tdrd5l* regulation indicates that a previously unidentified signal from the somatic gonad to the germline regulates sex-specific germline gene expression and we are currently studying the nature of this signal.

1900F **A fitness analysis of *OdsH* in *Drosophila* using selection with two alleles of X-linkage** Sha Sun[1,2], Chau-Ti Ting[2,3], Chung-I Wu[2,4] [1]Univ California, Irvine, [2]University of Chicago, [3]National Taiwan University, [4]Sun Yat-sen University

In organisms with the XY sex-determination system, there is an imbalance in the inheritance and transmission of the X chromosome between males and females. Unlike an autosomal gene, an X-linked recessive gene in a female will have phenotypic effects on its male counterpart. Thus, genes located on the X chromosome are of particular interest to researchers in molecular evolution and genetics. Here we present a model for selection with two alleles of X-linkage to understand evolutionary forces acting on genes on the X chromosome. This model was applied to the fitness analysis of an X-linked gene, OdsH, in the fruit fly *Drosophila melanogaster*. We introduced a site-specific sequence modification into the *OdsH* locus that was easily identified by an eye color marker gene. In a laboratory population setting, after twenty generations of fitness competition, two genetically modified *OdsH* variants displayed a 40% difference in allele frequencies. Using maximum likelihood estimation (MLE), we determined the fitness components of the *OdsH* alleles separately in males and females,

including viability and fertility effects. Results of the analysis indicate that the two *OdsH* gene variants show a fitness difference, and that sex-specific fertility and viability effects are both contributing factors to selection on an X-linked gene.

1901F **Transcriptomics analysis of allergen-induced inflammatory gene expression in the Four-Core Genotype mouse model** Carolyn Damilola Ekpruke[1], Rachel Alford[1], Dustin Rousselle[1], Maksat Babayev[1], Shikha Sharma[1], Sarah Commodore[1], Aaron Buechlein[2], Douglas Brandt Rusch[2], Patricia Silveyra[1] [1]Environmental and Occupational Health, Indiana University Bloomington, [2]Indiana University Bloomington

Sex differences in allergic inflammation have been reported, but the mechanisms underlying these differences remain unknown. Contributions of both sex hormones and sex-related genes to these mechanisms have been previously suggested in clinical and animal studies. Here, Four Core Genotypes (FCG) mouse model was used to study the inflammatory response to house dust mite (HDM) challenge and identify differentially expressed genes (DEGs) and regulatory pathways in lung tissue. Briefly, adult mice (8-10 weeks old) of the FCG (XXM, XXF, XYM, XYF) were challenged intranasally with 25µg of HDM or vehicle (PBS-control group) 5 days/week for 5 weeks (n=3/10group). At 72 hours after the last exposure, we analyzed the eosinophils and neutrophils in the bronchoalveolar lavage (BAL) of FCG mice. We extracted lung tissue and determined DEGs using Templated Oligo-Sequencing (TempO-Seq). DEGs analysis was performed using the DESeq2 package and gene enrichment analysis was done using Ingenuity Pathway Analysis. A total of 2,863 DEGs were identified in the FCG. Results revealed increased eosinophilia and neutrophilia in the HDM-treated group with the most significantly expressed genes in XYF phenotype and a predominant effect of female hormones vs. chromosomes. Regardless of the sex hormones, mice with female chromosomes had more downregulated genes in the HDM group but this was reversed in the control group. Interestingly, genes associated with inflammatory responses were overrepresented in the XXM and XYF genotypes treated with HDM. Sex hormones and chromosomes contribute to inflammatory responses to HDM challenge, with female hormones exerting a predominant effect mediated by inflammatory DEGs.

1902F **Loss of a postmeiotically expressed X to autosome retrogene, *Phf8l*, results in male subfertility** Ivan F Mier, Martin F Arlt, Jacob L Mueller Human Genetics, University of Michigan

X to autosomal retrogene copies are primarily thought to compensate for meiotic sex chromosome inactivation (MSCI), the silencing of X- and Y-linked genes. Here we report a copy of the X-linked gene *Phf8,* that retrotransposed to an autosome in *Muridae* (*Phf8l)* and acquired postmeiotic-specific expression. In *Peromyscus*, *Phf8l* transposed to the Y Chromosome (*Phf8y)*, massively amplified (~140 copies), and retained postmeiotic-specific expression. The specific expression of *Phf8l,* and *Phf8y* in postmeiotic haploid sperm cells suggests these lineage-specific genes have a role in male fertility. Using CRISPR/Cas9 we generated *Phf8l* $^{-/-}$ mice, and used existing *Phf8* $^{-/Y}$ mice, to study the roles of *Phf8l* and *Phf8* in male fertility. *Phf8l* $^{-/-}$ mice have reduced fecundity. In *Phf8l* $^{-/-}$ *Phf8* $^{-/Y}$ mice, 5S rRNA gene expression is highly upregulated in the testis, indicating PHF8 and PHF8L potentially repress postmeiotic 5S rRNA transcription. We find PHF8L directly interacts with FAM178b and ZFP318, which may facilitate target 5S rRNA repression. Our studies of *Phf8l* and *Phf8* highlight the importance of studying autosomal retrogenes with postmeiotic gene expression in addition to those that compensate for MSCI.

1903F **The Integration Institute: Sex, Aging, Genomics, and Evolution (IISAGE)** Ellie Duan[1], Peggy R Biga[2], Anne Bronikowski[3], Tony Gamble[4], Erica Larschan[5], Richard Meisel[6], Ritambhara Singh[5], James Walters[7], Ashley Webb[8], Gerald Wilkinson[9], Nicole Riddle[2] [1]Cornell University, [2]The University of Alabama at Birmingham, [3]Michigan State University, [4]Marquette University, [5]Brown University, [6]University of Houston, [7]The University of Kansas, [8]buck institute, [9]University of Maryland

In many animals, aging shows sex-specific patterns, often with one sex aging faster or having a shorter lifespan. What causes the diverse patterns of sex-specific aging across the animal kingdom is unknown. The IISAGE Biology Integration Institute will determine how diverse biological processes contribute to sex differences in aging and uncover their evolutionary history. IISAGE will bring together expertise from across biology to identify the molecular mechanisms and generalizable rules that govern differences in aging between females and males. We will test hypotheses focused on differences between females and males in genome architecture, organismal biology, and phenotypic plasticity to understand differences in aging. IISAGE will define how processes at the molecular, organismal, and population level interact to generate sex differences in aging. IISAGE will produce novel analysis tools and hundreds of matched datasets profiling gene expression and chromatin in dozens of species, including several Drosophila species, houseflies, and lepidoptera. By integrating across disciplines, approaches, and levels of biological organization, IISAGE will develop predictive models for how genome architecture, organismal biology, and phenotypic plasticity can interact and lead to differences in aging. Integrated with its scientific mission, IISAGE's training, education, and outreach program will increase diversity in STEM and prepare trainees to work in diverse careers and in multidisciplinary teams. The IISAGE summer program will engage > 50 undergraduates from groups underrepresented in STEM. A citizen science project will engage pet owners and K-12 students to collect data for IISAGE scientific goals.

1904F **Exploring the hippocampal transcriptome following perturbations of locally synthesized estrogen in the zebra finch (Taeniopygia guttata)** Rebecca Andrade[1], Yvette M Nau[2], Zoe Joy[1], Elaina Bashaw[1], John Bracht[2] [1]Neuroscience, American University, [2]Biology, American University

The conversion of testosterone, which circulates at high levels in males, into estradiol (E2), which circulates at high levels in the female, via aromatization occurs in many tissues including the brain in several vertebrates. In songbirds, the hippocampus (HP) a telencephalic structure critical for learning and memory, is a major site of E2 synthesis where aromatase is readily detectable in both sexes. In the HP, aromatase is abundant at synaptic loci and is surprisingly sparse in other neuronal compartments. Decreases in local E2 synthesis via the aromatase inhibitor 1,4,6-Androstatriene-3,17-dione (ATD) in the zebra finch (*Taeniopygia guttata*) HP impairs spatial memory performance such as seen in HP-lesioned birds, Aromatase inhibition with concomitant E2 replacement restores memory function back to control levels, suggesting that local synaptic aromatization may be a crucial modulator of memory function in songbirds. The genomic mechanisms underlying these observations, however, are unknown. This project seeks to understand the transcriptional changes that contribute to the poor spatial memory performance in male and female zebra finches lacking E2 in the HP by studying three hippocampal treatment groups: positive control, ATD, and ATD with E2 replacement. Using weighted gene correlation network analysis (WGCNA), the project centers around identifying gene clusters within RNAseq data (Illumina) across the treatment groups outlined above, revealing genes that systematically change under variations in the endocrine environment through the presence or absence of E2.

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1905S **Early-life exercise in Drosophila alleviates the sex-specific divergence of aging-associated gene expression in thorax muscle tissue** Eric C Randolph[1,2], Peggy R Biga[1], Nicole C Riddle[1] [1]Biology, University of Alabama at Birmingham, [2]University of Alabama at Birmingham

Transcriptional differences associated with aging are common in muscle tissue. Muscles also alter transcription in response to exercise irrespective of age. Exercise therefore has the potential to affect transcriptional differences associated with aging in muscle tissue. The optimal intensity, duration, frequency, and age at which exercise has the greatest effect on aging-related transcriptional differences are currently unknown. Available data suggest that there might be sex-specific responses to exercise. *Drosophila melanogaster* is an ideal model to investigate exercise effects as Drosophila are sexually dimorphic, have a short lifespan, are commonly used to study molecular mechanisms of aging, and share many physiological responses to exercise with humans. This study investigates sex-specific differences in transcriptional responses to early-life exercise in Drosophila muscle tissue. We will determine whether these gene expression responses persist to later ages or influence transcriptional differences associated with aging in adult flies.

We collected transcriptome data from wild-type male and female Drosophila that were either non-exercised or exercised for 2 hours/day for 5 days early in life. Flies were sampled at two time points, young (8 days) and old (54 days). Transcriptome analysis focused on the thorax to define gene expression differences in muscle tissue between sexes in both age and exercise. A principal component analysis indicated a clear separation of gene expression profiles between sexes along the PC1 axis, indicating sex as the largest determinant in gene expression differences. Thorax tissue from non-exercised flies showed 244 differentially expressed genes (DEGs) associated with aging in females, 189 DEGs in males, and 52 shared DEGs indicating the presence of sex-specific transcriptional differences associated with aging. Directly after early-life exercise, thorax muscle tissue showed 116 DEGs associated with exercise in females, 39 DEGs in males, and 5 shared DEGs indicating sex-specific responses to exercise. DEGs associated with aging were reduced more than half in thorax tissue from exercised files with females showing 84 DEGs, 87 DEGs in males, and 10 shared DEGs. Thus, early life exercise in Drosophila appears to reduce sex-specific transcriptional differences associated with aging in thorax tissue. Studying gene regulation differences between sexes during aging provides insights into genetic influences on sex-specific aging.

1906S **Genetic control of mating plug ejection timing in *Drosophila melanogaster*** Rachel M J Craig, Bianca M Villanueva, Jolie Carlisle, Adriana Andrus, Mikaela Matera-Vatnick, Dawn S Chen, David Cabello, Andrew G Clark, Mariana F Wolfner Cornell University

Although it would seem that males and females both benefit by cooperating to maximize offspring number, competition between male and female reproductive strategies can lead to sexual conflict, which in turn can drive rapid diversification of genes mediating reproductive processes. In *Drosophila*, female flies can manipulate the parental contributions of males by controlling the number of sperm stored from a mating. The “mating plug” (MP), which forms in the uterus of the female via coagulation of ejaculated seminal fluid proteins (Sfps) and four female proteins, is a contributor to this process. Rapid ejection of the MP prevents sperm retention, while delayed ejection of the MP increases the number of sperm that can be stored. However, nothing is known about the molecules (male or female) that modulate MP ejection (MPE) rates, and how those

molecules may interact to control that modulation. Using the *Drosophila* Genetic Reference Panel, we measured the mean times of MPE across many female genetic backgrounds. We then performed a genome wide association study to identify candidate genes that may influence MPE, which revealed a large number of neuronal genes and genes involved in oogenesis. We are beginning to test the neuronal candidate genes by using RNAi KD to determine their role in mating plug ejection. Additionally, we determined using neural network predictions that several of the neuronal candidates may be in a network with each other. Using daughters of Tudor flies, which lack a germline, compared to genetically matched flies with a germline, we were able to determine that oogenesis influences MPE timing as well, and we have begun to investigate this connection further using other known oogenesis mutants. Finally, we have begun to genetically examine the roles of MP Sfps on MPE timing.

1907S **Geographic origin and sleep patterns predict disease outcomes in *Drosophila melanogaster*** Mintong Nan[1], Jonathan Wang[2], Raymond St. Leger[1] [1]Entomology, University of Maryland, [2]Sarepta Therapeutics

Given the diversity in disease outcomes, even among closely related individuals, there is an urgent need to examine how defense is linked with other aspects of host physiology. The overall goal of this study was to employ *Metarhizium anisopliae* 549-*Drosophila* as a model system to examine how environmental and genetic factors influence fly survival across different biomes, as well as interactions between fungal infection, sleep, and host defense. We showed that resistance to Ma549 varies by sex and place of origin, with males and African lines usually being more resistant, and correlated with sleep parameters. Both the mean climate and climate extremes at collection sites, particularly tropical sites, were associated with disease resistance in the laboratory. Using representative fly lines, we found that daytime sickness sleep increased within the first two days post infection with Ma549, particularly in males of a line that were very resistant. Host defenses are linked to circadian rhythms in living organisms. Disrupted circadian rhythm can thus influence disease outcomes. Here, we employed a well-characterized sleep-deprived *Drosophila Shaker* mutant with intact circadian rhythms to confirm that sickness sleep contributes to enhanced survival. We also used two mutants with disrupted circadian clock-controlled genes to examine the role of the clock in activity and sickness sleep after Ma549 infection. Our data demonstrate that *period* and *Shaker* genes protect against disease, whereas active *ClkJrk* reduces survival in infected *Drosophila*.

1908S **Male Meiotic X Chromosome Inactivation in X-A Translocations and its Implications for Sperm Viability in *Drosophila*** Camila C Avelino[1,2], Timothy L Karr[1], Maria D Vibranovski[2,3] [1]Biodesign Institute, Arizona State University, [2]Department of Genetics and Evolutionary Biology, University of Sao Paulo, [3]School of Mathematical and Natural Sciences, New College of Interdisciplinary Arts and Sciences, ASU, Arizona State University

Chromosomal translocations between the X chromosome and autosomes frequently underlie male infertility in various species, including *Drosophila.* This phenomenon is closely linked to the regulatory process known as Meiotic Sex Chromosome Inactivation (MSCI). Our recent research has revealed that the loss of RNA polymerase II activity during the final stages of meiosis I on the X results in their inactivation. However, it remains uncertain whether this inactivation is chromosomally associated or if it is tied to a specific nuclear territory where these chromosomes are recruited to. In an effort to investigate those hypotheses and understand the causes of male infertility in *Drosophila*, this study employed *Drosophila melanogaster*'s lineages bearing X-autosomal 2nd chromosome translocations, both fertile and infertile, to examine the transcriptional activity of translocated chromosomes at different spermatogenic stages and to analyze their impact on sperm structures. Utilizing multi-color fluorescent in situ hybridization (FISH) probes in *Drosophila* brain cells, our investigation revealed that sterile males exhibited reciprocal translocations involving the euchromatic regions of the X and 2nd chromosomes. Conversely, within the fertile lineage, we observed non-reciprocal translocation, with the euchromatic region of the 2nd chromosome relocating to the X chromosome, leaving the latter chromosome intact. In male meiotic cells of sterile lineages, the translocated portion of the X chromosome was found within the autosomal territory, indicating that male infertility is a consequence of regulatory changes deriving from the X chromosome›s relocation to a different chromosomal territory. In contrast, the other relocation involving the autosomal 2nd chromosome did not produce the same effect, as confirmed through double immunofluorescence-FISH analysis. We also found in the late spermatogenic stages the fertile strain had multiple compacted spermatid bundles and individualization complexes whereas in the infertile strains, there were scattered spermatid nuclei and a few Individualization complexes, besides no mature sperms in the seminal vesicle. This study elucidates that X chromosome inactivation is subject to territorial-level regulation, and the recruitment of the X chromosome into an autosomal territory significantly impacts male fertility.

1909S **Mass spectrometry revealed sexual dimorphism in response to cancer** Anindita Barua[1], Fei Cong[1], Yang Tang[2], Xiaowen Liu[2], Wu-Min Deng[1] [1]Biochemistry and Molecular Biology, Tulane University, Louisiana Cancer Research Center, [2]Biomedical Informatics and Genomics, Tulane University, New Orleans Bioinnovation Center

Sex disparities in cancer incidences and mortality is an ongoing quest. Genetic mutation leading to cancer, responses to

carcinogens, cancer prognosis or diagnosis, response, or resistance to chemotherapy, all controlled in a sex-specific manner. It necessitates exploring the key factors and signaling pathways fueling the sexual dimorphism in cancer. Our study uses the *Drosophila* tumor model to investigate the sex-specific response to cancer and how it contributes to changing signaling pathways, thus enhancing tumor growth. Previously, our lab induced primary tumors in the larval salivary gland transition zone (TZ) by constitutively expressing the Notch intracellular domain (NICD) under the retn-gal4 driver. In the current study, we injected the primary tumor in the w^{1118} fly abdomen, where the tumor proliferates and develops into a first-generation tumor mass. *Drosophila* lifespan depends on many factors, and tumor injection causes aging and a significant lifespan reduction. Generally, a control female without a tumor lives ~ 49 days, and a control male lives ~ 43 days. However, with tumors, both sexes live ~32 days, a comparable mortality age. It indicates a disproportionately increased aging in female tumor hosts compared to male, showing sex-specific differences in cancer/tumor response. To investigate the differences at the proteomic level, we performed mass spectrometry (MS) on the whole fly tissue collected after ten days of tumor injection. We removed the tumor from the host abdomen prior to MS. MS identified 834 differentially expressed proteins (DEPs) between the tumor injected and control female, but only 539 DEPs for males. The data further reveals only 166 upregulated and 71 downregulated DEPs shared between males and females, thus indicating a distinct sex-specific response to tumors in the *Drosophila* model. The Gene ontology (GO) reveals DEPs to be associated with metabolic and innate immune pathways, pointing to a possible area to focus on for elucidating the differential responses to cancer based on sex.

1910S **Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the Somatic Gonad** Natalie A Murphy, Samantha C Goetting, Jose Pac Cordero, Mark Van Doren Biology, Johns Hopkins University

Doublesex (Dsx) and Mab-3 Related Transcription factors (DMRTs) are paramount for sex determination across the animal kingdom. In flies, Dsx, like mammalian Dmrt1, is first expressed in the somatic gonad during embryogenesis, and is required for male vs. female gonad development. There are two Dsx isoforms – Dsx^F in females and Dsx^M in males – which have the same DNA binding domain but regulate their targets differently to yield sexual dimorphism. Bioinformatic analyses were used to predict Dsx targets; many of the top hits were transcription factors containing BTB (Broad-complex, Tramtrack, and Bric-a-brac) domain(s), which promote homo- and hetero-dimerization of BTB proteins. BTB domain transcription factors are important for gonad development in both the male and female, including *bab1/2*, *chinmo*, *fru*, and *mamo*. An *RNAi* screen of BTB domain transcription factors recapitulated these results and revealed another factor worth further characterization: *lola* (*longitudinals lacking*).

lola has a multitude of differentially spliced isoforms and has many putative Dsx binding sites along the entire length of the gene. Previous studies have shown isoform-specific roles in proper somatic gonadal precursor (SGP) coalescence during embryonic gonad formation as well as roles in adult somatic niche cells (terminal filaments/cap cells and cyst stem cells, in the females and males, respectively). We are curious if there are additional sex-specific roles and/or expression of certain *lola* isoforms. In the larval ovary, *lola* knock-down via the somatic driver c587 caused disorganization of the intermingled cells and the terminal filaments – interestingly, this phenotype appears to be a combination of both *bab* and *mamo* phenotypes. In the larval testis, *lola* knock-down produced an expanded “DAPI bright” zone of less differentiated germ cells. However, there appeared to be a decrease in the number of less differentiated (i.e., zfh1-positive) cyst cells/cyst stem cells.

Given these data, we hypothesize that *lola* is regulated by Dsx and plays an important role in both male and female somatic cell fate. It may be interacting with other BTB domain proteins (*mamo* and *bab1/2* in the female; *chinmo* in the male) to create the sexual dimorphism of the ovary and testis. Ultimately, we aim to expand the current knowledge on how DMRTs control sexual dimorphism and elucidate genetic interactions critical for sexual identity in both flies and mammals.

1911S **Investigating the spreading mechanism of the MSL Dosage Compensation Complex on the X chromosome.** Arthur Langford[1], Mitzi I Kuroda[2,3], Janel Cabrera[1] [1]Biology, Emmanuel College, [2]Medicine, Brigham and Women’s Hospital, [3]Genetics, Harvard Medical School

While previous research has made progress in characterizing the specific proteins that participate in gene activating complexes, the overall mechanism of how they interact with chromatin targets to induce gene activation is not well understood. Therefore, to further investigate their behavior, we utilized the Dosage Compensation Complex (DCC) as a model for studying interactions between gene activating complexes and their gene targets. The DCC is responsible for upregulating transcription of X-linked genes in male Drosophila by two-fold during dosage compensation to compensate for the dosage differences of such genes between males and females. There are two main theories proposed for how the DCC carries out dosage compensation: a sequence-dependent or sequence-independent manner. To determine the course of action taken by the DCC during dosage compensation, we developed Drosophila mutants lacking copies of the endogenous MSL2 gene, which encodes for the MSL2 DCC protein component, and delivered MSL2 back to such flies at gradual time points during early

development using a heat shock/FLP-FRT recombinase system. In this fly system, the spread of the DCC on chromatin can now be analyzed using immunostainings and in situ hybridizations. We hypothesize that the DCC carries out dosage compensation and spreads across the X chromosome in a sequence-independent manner. This model can be used to investigate the potential method of action taken by regulatory protein complexes in order to activate genes and offer insight on how they may become compromised in human diseases.

1912S **Knockout of highly conserved SFP ablates male fertility** Dominic Hockenbury[1], Yasir Hashim Ahmed-Braimah[2] [1]Center for Reproductive Evolution, Syracuse University, [2]Center for Reproductive Evolution, Biology, Syracuse University

In *Drosophila,* seminal fluid proteins (SFPs) are key components of the male ejaculate and are essential determiners of reproductive fitness. SFPs are required for fertility as well as inducing a range of post mating physiological responses in females. To date nearly 300 SFPs have been identified, however the vast majority of these remain uncharacterized and their role in reproduction and/or postcopulatory sexual selection is not clear. SFPs are functionally diverse and contain proteins from a variety of biochemical processes, most notably proteases and protease inhibitors that are thought to be critically important in biochemical interactions with female proteins. Another class of proteins that is enriched among SFPs are carbohydrate bonding proteins, presumably involved in glycolytic reactions within the accessory glands and in mated females. However, the molecular mechanisms and fertility roles of these ejaculate glycolysis modulators have yet to be explored. One of these uncharacterized glycolysis SFPs is the highly conserved *beta*-glucoronidase (*CG15117*), which catalyzes the breakdown of complex carbohydrates. *CG15117* is (1) predominately expressed in the male accessory gland—but maintains low level expression in other tissues, (2) is transferred to the female during mating, and (3) is one of the most conserved SFPs in *Drosophila.* To identify CG15117's role in reproduction, we created a CRISPR/Cas-9 knockout mutant by ablating 5 bp from the coding sequence and introducing a premature stop codon. We found that CG15117 is required for male fertility: females that are mated to knockout males do not produce progeny, fail to store sperm and will readily remate, suggesting that an ensemble of key postcopulatory processes is disrupted. To further examine the molecular basis of this male sterility we performed label-free quantitative proteomic analysis and bulk RNA sequencing on mutant males as well as females mated to mutant and control males and find systematic abnormal abundance of several proteins—including some SFPs— in the accessory glands and in the transferred male ejaculate. Our results show that the often-ignored carbohydrate metabolism proteins that are part of the seminal plasma are essential for fertility in *Drosophila*.

1913S **Temporal transcriptomics identifies sexually dimorphic trajectories during mouse adrenal gland postnatal development** Ben Maggard, Yuan Kang, Hui Wang, Chen-Che Jeff Huang Auburn University

The primary aim of this research is to elucidate the genetic expression patterns throughout the postnatal development of the mouse (Mus musculus) adrenal gland at a genome-wide level. Employing RNA sequencing (RNA-seq) coupled with bioinformatics analysis, we aim to identify potential genes and pathways steering the maturation of this pivotal endocrine organ. RNAs from whole adrenal glands in wild-type mice were extracted at postnatal days (P) 0, 7, 15, 21, 28, 35, and 49, with a minimum of three data points analyzed at each developmental stage. Each data point comprises the adrenal glands of 3-4 mice, and the extracted RNAs underwent paired-end RNA-seq analysis through a commercial service provider. Results revealed a notable increase in the expression of most differentially expressed genes (DEGs) from P0 to P7. The construction of a heatmap encompassing all DEGs unveiled gene clusters contributing to the sexual dimorphism observed in the adrenal gland transcriptome during early postnatal development. Notably, genes within these clusters exhibited an age-dependent sexual dimorphism commencing from P28. Gene ontology analysis indicated that, at P0, DEGs between males and females were associated with gene expression regulation, particularly histone demethylation. Conversely, DEGs at subsequent developmental stages were linked to the specific functions of the adrenal gland. Notably, many of these gene ontology terms were related to lipid metabolism, encompassing processes such as steroid biosynthesis, fatty acid metabolism, and monocarboxylic acid catabolism. This suggests the presence of a sexually dimorphic lipid metabolism in the mouse adrenal gland.

1914S **Consequences of variable escape from X inactivation in humans** Carrie Zhu, Jared Cole, Arbel Harpak The University of Texas at Austin

In humans, X chromosome inactivation substantially reduces gene expression from the "inactive" X (Xi). While inactivation is usually thought of as absolute—with escape from inactivation being a rare exception—recent work exposed surprising variation in Xi expression levels across genes, tissues and individuals. We investigated the consequences of variation in Xi expression for sex differences and within-sex variation.

First, we show that in non-recombining regions, and across 13 tissues examined, Xi expression levels estimated from a single individual linearly predict sex differences in gene expression remarkably well. We conclude that continuous variation in Xi

expression is the major driver of sex differences in expression in the non-recombining X, with only a minor role for variation in Xi expression among individuals and compensatory regulatory mechanisms. In contrast, in the pseudoautosomal region, Xi expression and sex differences were entirely uncorrelated.

Second, we show that variation in Xi expression across genes has a surprisingly small effect on variation in gene expression within females. We develop theoretical predictions for the contribution of X-linked regulatory genetic variation to heritability differences between males and females, and fit it to eQTL and GWAS data in genes of variable Xi expression. With few exceptions, the strong fit to the data further supports a small role of other compensatory mechanisms.

Overall, our results demonstrate that the continuous variation in Xi expression is a key factor underlying sex differences in humans.

1915S **Understanding Sex Determination in African Clawed Frogs (*Xenopus)*** Sarah Porter[1], Lindsey Kukoly[2], Caroline Cauret[2], Danielle Jordan[3], Benjamin Evans[2], Richard Behringer[4], Marko Horb[1] [1]Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, [2]Biology, McMaster University, [3]School of Biological Sciences, University of Aberdeen, [4]Genetics, University of Texas MD Anderson Cancer Center

In amphibians there is great diversity among species in the genetic mechanisms by which sex determination is achieved. For example, considerable variation exists in whether males or females are heterogametic (and thus whether there are XY/XX or ZW/ZZ sex chromosomes). In African clawed frogs (*Xenopus*), all known species have female heterogamy, however the location of the sex chromosomes varies significantly. *Xenopus* species lack diverged sex chromosomes and instead possess sex determining loci located on different autosomes. Most *Xenopus* species display a ZZ/ZW system, where the female is the heterogametic sex. Within the *Xenopus* genus, we focused on two species: *X. laevis* and *X. tropicalis*. *X. laevis* are tetraploids, which possess L and S allo-alleles. The sex determining locus for this species has been identified as W chromosome and is located on chromosome 2L. Located within this region, the gene *dm-w*, determines female sex by inhibiting the expression of dmrt1(which is responsible for male sex determination). Comparatively, *X. tropicalis* is a diploid organism, where chromosome 7 is believed to possess the sex determining loci. X*. tropicalis* lacks *dm-w* altogether and it is unknown if there is a single sex determining gene responsible for the female sex in this species. I will present our efforts of using CRISPR-Cas9 techniques to study the mechanistic aspects of how genetic sex determination functions and evolves in *Xenopus*. Moreover, several other *Xenopus* mutants have been generated (*amh, foxl2, dmrt1, ccdc69w, scanw, dmw & ar)* to assess their involvement in sex determination. We aim to use knockout mutants and gene expression techniques to develop a comparative framework in which to better understand how the genetic basis of sex determination systems have evolved and function within the model species, *Xenopus*.

1916S **Sexually dimorphic Atf4 expression and activity control adipose tissue physiology** Lydia Grmai[1], Melissa Mychalczuk[2], Aditya Arkalgud[1], Deepika Vasudevan[1] [1]Cell Biology, University of Pittsburgh, [2]Biochemistry, Molecular, and Cell Biology, Cornell University

Metabolic differences between males and females have been well documented across many species. However, the molecular basis of these differences and how they impact tolerance to nutrient deprivation is still under investigation. In this work, we use *Drosophila melanogaster* to demonstrate that sex-specific differences in fat tissue metabolism are driven, in part, by dimorphic expression of the Integrated Stress Response (ISR) transcription factor, Atf4. We found that female fat tissues have higher Atf4 activity than their male counter parts under homeostatic conditions. This dimorphism was partly due to a female bias in *Atf4* transcript abundance in fat tissues and driven by canonical sex determinants: masculinization of female fat tissues via depletion of the sex determinant *transformer* alters the relative abundance of *Atf4* splice isoforms. These differences persist under stress conditions, where female fat tissues show substantially higher Atf4 induction than males in a genetic model of nutrient deprivation, indicating that higher Atf4 activity confers higher tolerance to nutrient deprivation in females. Finally, we demonstrate that dimorphic ISR activity in fat tissues confers a metabolic advantage that increases stress tolerance into adulthood: genetic induction of nutrient deprivation caused developmental lethality specifically in males. We are currently testing the dependence of this sex-specific viability defect on ISR pathway activation. Together, our data describe a previously unknown facet of ISR signaling wherein sexual identity of adipose tissue confers differential stress tolerance in males and females. Future work will investigate functional differences between Atf4 isoforms and how they inform sex differences in fat tissues. Since Atf4 promotes cellular function and survival under homeostasis and stress, our studies will shed light on how sexual identity influences metabolism in both homeostasis and disease.

1917V **The between-sex multivariate genetic architecture acts as a constraint to the evolution of sex-difference in contemporary humans** Anasuya Chakrabarty[1], Saikat Chakraborty[2], Diptarup Nandi[3], Analabha Basu[1] [1]National Institute of Biomedical Genomics, [2]GSK India, [3]Azim Premji University

Sex-difference (SD) is ubiquitous in humans despite a shared genetic architecture between the sexes. In human genetics, SD is usually studied by estimating the between-sex genetic correlation (r_{mf}) of traits (e.g., correlation between height in males and females). But inspecting SD in single traits does not provide a complete biological overview, because traits are not independent and are genetically coupled. One way to circumvent this is to investigate the multivariate genetic architecture of the sexes by estimating the multivariate counterpart of r_{mf}, the **B** matrix which contains the between-sex genetic covariances in the diagonal and the between-sex-between-trait genetic covariances (e.g., covariance between height in males and weight in females) in the off-diagonals. **B** is indirectly shaped by sex-specific selection and is decisive in the evolution of SD as it can constrain or facilitate the independent divergence of the sexes. Though investigating **B** is common in other animals, there has been a dearth of such studies in humans. Using such a multivariate approach, we investigated SD in the genetic architecture of 12 anthropometric, fat depositional, and sex-hormonal phenotypes in humans from publicly available GWAS summary statistics of the UK Biobank under a LD score regression framework. We found that the sexes not only have a different genetic architecture, but intriguingly the directions of most of the between-sex-between-trait covariances were opposite, most prominently between testosterone and the anthropometric traits. Testosterone is closely associated with fitness in males, and BMI (anthropometric) is an indicator of obesity which has ramifications in many complex disorders. We estimated an overwhelming number of negative genetic correlations, 28 out of 66 pairs in between-sex-between-trait, which indicates sexual antagonism in humans. We found **B** to be asymmetric which indicates difference in the underlying genetic architecture between the sexes. Furthermore, **B** did not act as a genetic constraint to the predicted divergence between the sexes in response to simulated concordant random selection gradients. In contrast, **B** substantially reduced the predicted divergence between males and females when randomised selection vectors were antagonistic. Though we found sexual antagonism in our study, the evolution of SD under sexually antagonistic selection in contemporary humans would be constrained by the multivariate between-sex genetic architecture.

1918V **Genetic and behavioural correlates of a male-specific aging phenotype in Drosophila hypocausta** Lauren Kovacik[1], Kevin Wei[2] [1]University of British Columbia, [2]University of Bristish Columbia

Drosophila has long been a model system for understanding the genetic and evolutionary mechanisms underlying sexual dimorphism in color patterns. We identified a unique sexual dimorphic phenotype in Drosophila hypocausta, in which the males dramatically darken as they age, while females remain pale. This species offers a unique system to understand the interaction between sexual dimorphism, sex-specific aging, and sexual selection. To uncover the genetic architecture of age- and sex-dependent coloration differences, we collected RNA-seq data from males and females of increasing age (3, 10, and >25 days old); male-specific gene expression either increased or decreased over time. Using various statistical and graphical techniques, 3 genes were identified that are significantly differentially expressed between the sexes and ages, including the body coloration gene yellow which shows a staggering 128-fold decrease in expression between young and old males. These genes are strong candidates that require further exploration, starting with a knockout of yellow using CRISPR-Cas9. To understand the purpose of this darkening phenotype, we hypothesize that it acts as a signal for sexual maturity thus conferring selective advantage in aged males through increased mating success. Supporting this possibility, we find that older and darker males consistently have higher mating success in female mate-choice assays. Additional behavioural assays are currently underway to differentiate between female preference versus male sexual maturity and elucidate other age-dependent behaviours.

1919T **Antifungal killer toxins from yeasts - discovery and their application in craft breweries.** Victor Zhong[1], Nicholas Ketchum[2], Ximena Garcia[1], Paul A Rowley[3] [1]University of Idaho, [2]Rhinegeist Brewery, [3]Biological Sciences, University of Idaho

Secondary fermentation of beer by diastatic strains of *Saccharomyces cerevisiae* can result in undesirable consequences, such as off-flavors, increased alcohol content, hyperattenuation, gushing, and the spontaneous explosion of packaging. Many strains of *Saccharomyces* yeasts can naturally produce proteinaceous “killer” toxins that inhibit the growth of competing yeasts. We recently completed a comprehensive study of killer toxin production by 1,270 *S. cerevisiae* strains and found that 50% produce killer toxins. This large collection of 638 diverse killer yeasts has enabled the discovery of killer toxins that can prevent the growth of diastatic yeasts. Specifically, 90% and 71% of diastatic strains are susceptible to the K1 and K2 killer toxins, respectively. This screening approach also identified a novel polymorphic K2 toxin that effectively inhibited K1-resistant diastatic yeasts. Adding killer yeasts to a simulated industrial-scale contamination event by diastatic yeasts successfully prevented hyperattenuation. Craft breweries can only safeguard against diastatic yeast contamination by using selective culturing methods or PCR to detect diastatic yeasts, followed by the destruction of contaminated products. Using killer yeasts in brewing offers a novel approach to safeguard against product loss and to remediate contaminated craft beers due to diastatic yeast contamination.

1920T **The Effect of Long Non-coding RNA Expression on Vitamin E Concentration in Maize Grain** Morgan A Apolonio[1], Sam Herr[2], Michael A Gore[2] [1]University of California, Berkeley, [2]Cornell University

Tocochromanols are a group of antioxidants synthesized in plant tissues and have varying levels of vitamin E activity, but tocochromanols with the highest vitamin E activity tend to occur at low levels in maize grain. In efforts to increase tocochromanol abundance in maize grain, Transcriptome-Wide Association Studies (TWAS) have been used to resolve quantitative trait loci (QTL) associated with grain tocochromanol concentrations to the gene level. While the causal loci underlying most of the large-effect QTL have been identified, relatively smaller effect QTL remain unresolved. Our past efforts have not explored the potential role of long non-coding RNAs (lncRNA), which are transcripts longer than 200 bp that do not encode a protein, in the genetic control of natural variation for tocochromanols in maize grain. Therefore, we hypothesize that lncRNAs could account for a portion of the unexplained genetic variation. We tested this hypothesis by conducting a new TWAS analysis in a maize association panel scored for lncRNA expression and grain tocochromanol levels. Nearly 20% of the identified lncRNAs were expressed in more than 10% of the individuals in the panel and were retained for the TWAS analysis. Of these, we found thirteen putative lncRNAs to be significantly associated with tocochromanols and of which four coincided with unresolved grain tocochromanol QTL identified in the maize nested association mapping panel. Our results highlight that including lncRNAs may be useful in resolving vitamin E-associated QTL in maize grain.

1921T **Breeding of high iron and zinc and grain yield under abiotic stress conditions, supporting enhanced maize biofortification** Tesfaye TW Mekonnen, Maryke MT Labuschagne, Angeline AV van Biljon Plant Sciences, University of the Free State

Breeding of high iron and zinc and grain yield under abiotic stress conditions, supporting enhanced maize biofortification

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Abstract

Maize is a crucial staple food crop in Sub-Saharan Africa, particularly for low-income households. However, the combined threats of heat and drought stress have significantly reduced the yield potential of biofortified maize, resulting in macro and micronutrient deficiencies among the population relying on maize-based diets. To address this issue, the study aimed to investigate the correlation between biofortified inbred lines and hybrid performance in terms of nutritional attributes, such as iron (Fe), zinc (Zn), and protein, under combined heat and drought stress conditions. The experiment was conducted at CIMMYT in Zimbabwe, using an α-lattice design with three replications. A total of 77 crosses and seven parents, chosen for their zinc, iron, and provitamin A traits, were evaluated under abiotic stress conditions. The findings revealed that the hybrid effect significantly impacted various nutritional attributes, including zein fractions, Zn, Fe, phytic acid, and grain yield. Under combined heat and drought stress conditions, the hybrids' Fe, Zn, and grain yield concentrations significantly decreased. However, zein fractions and phytic acid content in the grains increased under stress conditions. There was a strong positive correlation between grain yield and the concentrations of Zn and Fe, particularly under combined stress conditions. This investigation confirmed that developing high-yielding maize varieties with enhanced micronutrient content is possible under combined heat and drought stress conditions. This finding is significant as it provides a potential solution to reduce micronutrient deficiencies in Sub-Saharan Africa. The study concluded that developing high-yielding maize varieties with increased micronutrient content is possible under combined heat and drought stress conditions. Furthermore, the positive correlation between grain yield and the concentrations of Fe and Zn suggests that hybrids.

Keywords: Drought, Fe, Heat, Maize, Protein, Zein fractions, Zn

1922F **Adaptation to hatchery conditions in the threatened westslope cutthroat trout (*Oncorhynchus clarkii lewisi*)** Ylenia Chiari[1], Leif Howard[2], Scott Relyea[3], James Dunnigan[3], Matthew C Boyer[4], Gordon Luikart[2] [1]Biology, George Mason University, [2]Flathead Lake Biological Station, Montana Conservation Genomics Laboratory, Division of Biological Science, University of Montana, [3]Sekokini Springs Hatchery, Montana Fish Wildlife and Parks, [4]Montana Fish Wildlife and Parks

Hatcheries play a crucial role as a substantial resource investment, offering a valuable tool to enhance angling opportunities and support the conservation of native fish. In addressing the imperative to conserve native fish, it becomes crucial to understand the degree to which the artificial production process affects survival and fitness of hatchery fish that are released into the wild. Hatchery may differ from natural environments in terms of crowding, light, temperature, diet, and competition, as well as exposure to pathogens and predators. Fish born and raised in hatchery until they reach a size suitable for release into the wild may adapt to the hatchery's specific conditions and potentially be maladapted to the natural environment where they are released. In this work, we have tested for adaptation to hatchery conditions in westslope cutthroat trout

(*Oncorhynchus clarkii lewisi*) and assessed if this adaptation persists once fish from the hatchery are re-introduced into the wild. To this purpose, we have genotyped through RAD-capture and obtained RNASeq from fish sampled from wild populations, fry generated from fish from these populations that were reared and spawned at Sekokini Springs hatchery, and from fish from the wild populations where the fry from the hatchery was released. We then tested for genetic (variation in allele frequencies obtained using more than 1000 SNPs) and epigenetic (differences in DNA methylation) adaptation affecting gene expression between natural, hatchery, and released fry. Our results will not only be useful for guiding conservation aquaculture practices at Sekokini Springs hatchery to enhance native trout restoration efforts, but will also further our understanding of rapid adaptation to captivity conditions.

1923F **Early-life fecal transplantation from high muscle yield rainbow trout to low muscle yield recipients accelerates somatic growth through respiratory and mitochondrial efficiency modulation.** Guglielmo Raymo University of Maryland

Previous studies conducted in our lab revealed microbial assemblages to vary significantly between high (ARS-FY-H) and low-fillet-yield (ARS-FY-L) genetic lines in adult rainbow trout. We hypothesized that a high ARS-FY-H donor microbiome can accelerate somatic growth in microbiome-depleted rainbow trout larvae of the ARS-FY-L line. Germ-depleted larvae of low ARS-FY-L line trout reared in sterile environments were exposed to high or low-fillet yield-derived microbiomes starting at first feeding for 27 weeks. Despite weight-normalized diets, somatic mass was significantly increased in larvae receiving high fillet yield microbiome cocktails at 27 weeks post-hatch. RNA-seq from fish tails reveals enrichment in NADH dehydrogenase activity, oxygen carrier, hemoglobin complex, gas transport, and respiratory pathways in high fillet-yield recolonized larvae. Transcriptome interrogation suggests a relationship between electron transport chain inputs and body weight assimilation, mediated by the gut microbiome. These findings suggest that microbiome payload originating from high fillet yield adult donors primarily accelerates juvenile somatic mass assimilation through respiratory and mitochondrial input modulation. Further microbiome studies are warranted to assess how increasing beneficial microbial taxa could be a basis for formulating appropriate pre-, pro-, or post-biotics in the form of feed additives and lead to fecal transplantation protocols for accelerated feed conversion and fillet yield in aquaculture.

1924F **Cross-species Regulatory Network Analysis Identifies FOXO1 and the Circadian Clock as Drivers of Ovarian Follicular Recruitment** Ashley Kramer[1], Alberto Berral González[2], Kathryn Ellwood[3], Shanshan Ding[3], Javier De Las Rivas[2,2], Aditya Dutta[3] [1]Animal and Food Science, University of Delaware, [2]University of Salamanca, [3]University of Delaware

The laying hen ovary undergoes a complex process of follicular development, comprising primordial, primary, pre-recruitment, and pre-ovulatory stages. Transition to the pre-ovulatory stage is vital for successful ovulation. Although several differentially expressed genes (DEGs) have been identified in these stages, understanding the master regulators orchestrating gene expression changes remains incomplete. Here, we employ master regulator analysis (MRA) to identify key transcription factors controlling DEGs in pre-recruitment and pre-ovulatory follicles. Samples for RNA-seq were obtained from these stages and DEGs were determined. Subsequently, an ARACNe network analysis integrating human ovarian TCGA data with hen ovarian DEGs was performed, which was consequently used in the MRA to identify key transcription factors (TFs) using VIPER. Notably, FOXO1, known for its role in apoptosis and cell cycle regulation, emerged as a critical TF affecting the WNT/beta-catenin pathway. BHLHE40 and CLOCK were identified as regulators of the circadian rhythm, critical for the egg-laying cycle. RT-qPCR validation confirmed the upregulation of BHLHE40 and CLOCK, implicating their roles in regulating the pre-ovulatory stage. The downregulation of FOXO1, FST, and other TFs in the pre-ovulatory stage indicated their involvement in follicular development and hormone regulation. This study provides key insights into the regulatory mechanisms of follicular development in laying hens, offering a comprehensive understanding of the pivotal role of FOXO1 in mediating apoptosis and the regulatory roles of BHLHE40 and CLOCK in circadian rhythms. Understanding these master regulators will be crucial for unraveling the complex molecular processes governing ovulation and reproductive success in laying hens.

1925F **Identification of genetic and environmental factors influencing aerial root traits that support biological nitrogen fixation in sorghum** Wilfred Vermerris[1,2], Emily S.A. Wolf[3], Saddie Vela[3], Jennifer Wilker[4], Alyssa Davis[1], Madalen Robert[5], Rafael Venado[4], Catalin Voiniciuc[5], Jean-Michel Ané[4,6] [1]Microbiology & Cell Science, University of Florida, [2]UF Genetics Institute, University of Florida, [3]Plant Molecular & Cellular Biology, University of Florida, [4]Bacteriology, University of Wisconsin, [5]Horticultural Sciences, University of Florida, [6]Agronomy, University of Wisconsin

Biological nitrogen fixation (BNF) refers to the process by which symbiotic microorganisms reduce nitrogen from the air to ammonium that they make available to their host plants. Crop plants able to obtain a portion of their nitrogen needs via BNF require less nitrogen fertilizer, which contributes to more sustainable agricultural practice. Select accessions of the cereal crop sorghum (*Sorghum bicolor* (L.) Moench) form mucilage-producing aerial roots that harbor nitrogen-fixing bacteria. We performed a genome-wide association study (GWAS) of the sorghum minicore, a collection of 242 landraces, and 30 accessions from the sorghum association panel (SAP) in two locations (Florida and Wisconsin), under a standard or

reduced level of fertilizer, with the goal of identifying loci associated with the number of nodes with aerial roots and aerial root diameter. Sequence variation near genes encoding transcription factors that control phytohormone signaling and root system architecture showed significant associations with these traits. In addition, the location had a significant effect on the phenotypes. Concurrently, we developed an F2 population from crosses between bioenergy sorghums and a landrace that produced extensive aerial roots to evaluate the mode of inheritance of the loci identified by the GWAS. Furthermore, the mucilage collected from aerial roots contained polysaccharides rich in galactose, arabinose, and fucose, whose composition displayed minimal variation among 10 genotypes and the two fertilizer treatments. These combined results illustrate the benefit of allele mining, support the development of sorghums with the ability to acquire a substantial portion of their nitrogen needs via BNF, and provide insights in the mechanisms of BNF in other grasses.

1926S **Bacillus amyloliquefaciens modulate sugar metabolism to mitigate arsenic toxicity in Oryza sativa L. var Saryu-52** Harshita Joshi, Shashank Kumar Mishra Microbial Technologies, CSIR-NBRI

In the current study, plant growth-promoting rhizobacterium Bacillus amyloliquefaciens SN13 (SN13) was evaluated for arsenic (As) toxicity amelioration potential under arsenate (AsV) and arsenite (AsIII) stress exposed to rice (Oryza sativa var Saryu-52) plants for 15 days. The PGPR-mediated alleviation of As toxicity was demonstrated by modulated measures such as proline, total soluble sugar, malondialdehyde content, enzymatic status, relative water content, and electrolytic leakage in treated rice seedlings under arsenic-stressed conditions as compared to the respective control. SN13 inoculation not only improved the agronomic traits but also modulated the micronutrient concentrations (Fe, Mo, Zn, Cu, and Co). The desirable results were obtained due to a significant decrease in the AsIII and AsV accumulation in the shoot (47 and 10 mg kg−1 dw), and the root (62 and 26 mg kg−1 dw) in B. amyloliquefaciens inoculated seedlings as compared to their uninoculated root (98 and 43 mg kg−1 dw) and shoot (57 and 12 mg kg−1 dw), respectively. Further, metabolome (GC-MS) analysis was performed to decipher the underlying PGPR-induced mechanisms under arsenic stress. A total of 67 distinct metabolites were identified, which influence the metabolic and physiological factors to modulate the As stress. The expression analysis of metabolism- and stress-responsive genes further proclaimed the involvement of SN13 through modulating the carbohydrate metabolism in rice seedlings, to enable improved growth and As stress tolerance.

Keywords
As stress; GC-MS; Gene Expression; Metabolism; PGPR

1927S **De novo transcriptome assembly of 7 California native plant species** Savanah M. Senn[1], Gerald Presley[2], Steven Carrell[3], Meika Best[1], Les Vion[1], Matthew Kostoglou[1], John Hsieh[1], Adrianna L. Bowerman[1], Karu D. Smith[1], Daila Melendez[1,4] [1]Agriculture, LA Pierce College, [2]Wood Science & Engineering, Oregon State University, [3]Center for Quantitative Life Sciences, Oregon State University, [4]Horticulture, Oregon State University

How do plants in semi-arid environments respond to biotic and abiotic stress? In this study, we created transcriptomic resources for *Quercus agrifolia, Eriodictyon crassifolium, Trichostema lanatum, Dendromecon rigida, Artemisia californica, Arctostaphylos glauc*a, and *Ribes malvaceum* from the Angeles National Forest to facilitate inquiry.

During May 2022, snap frozen plant tissue samples for RNA sequencing were collected. Library prep was performed by BGI Americas and samples were sequenced on the DNBSeq platform. QC was performed with SOAPnuke. The trancriptomes were assembled in Trinity and assessed with BUSCO. Transcripts were quantified with salmon, and Trinotate was used for annotation. DE analysis was carried out in R DESeq2 for replicated *Quercus agrifolia* samples in different fire history areas of the Gold Creek Preserve.

The most complete assemblies were *Quercus agrifolia* (96.18% complete), *Dendromecon rigida* (95.44% complete), and *Eriodictyon californicum* (94.88% complete).

In oak tree leaves, there were elevated transcripts for myrcene synthase on the Red Trail and elevated geraniol-8 hydroxylase activity on the Green Trail. Elevated amounts of secondary metabolites could be influenced by light, temperature, herbivores, pathogens, or abiotic stress.

In the contrast between oak gene expression on the Green Trail vs. the Blue Trail, there was elevated myrcene synthase and isoprenoid biosynthetic activity on the Blue Trail, and elevated elevated triterpenoid synthesis activity on the Green Trail. It would be expected from the literature that the elevated isoprenoid biosynthetic process would occur in the most sun-exposed area of the Green Trail, however the transcripts related to the biosynthetic process were elevated on the Blue Trail.

Regarding antioxidants, in the contrast of the Red Trail vs. the Green Trail, Shikhimate/ Quinate dehydrogenase was higher on the Green Trail. In the contrast between the Green and Blue Trail, transcripts for Phylloquinone biosynthetic process were higher on the Green Trail. In the contrast between the Red Trail and Blue Trail, hydroquinone biosynthetic process transcripts

were higher on the Blue Trail, whereas Shikhimate/ quinate dehydratase and 3-dehydroxyquinate dehydratase/ shikhimate dehydrogenase transcripts were abundant on the Red Trail.

In the contrast between oak gene expression on the Red Trail vs. Green Trail, Malectin-like domain transcripts were higher on the Green Trail. Adenylate cyclase/ putative disease resistance protein was higher in oak transcripts from the Red Trail. Phosphatidylinositol phospholipase C /response to bacterium was higher on the Red Trail.

Our findings indicated that in *Quercus agrifolia,* there was differential expression of genes related to plant defense, antioxidants, and terpene synthesis. Elevation and the presence of potential pathogens are expected to be contributing factors.

1928S **Genetic characterization of a wild emmer by hard winter wheat backcross population** John Hill Price[1], Mary J Guttieri[1], Eduard Akhunov[2], Moses Nyine[2] [1]HWWGRU, USDA-ARS, [2]Dept. of Plant Pathology, Kansas State University

Wild emmer (*Triticum turgidum* subsp. dicoccoides), the tetraploid progenitor of hexaploid bread wheat (*Triticum aestivum*), is an important source of biotic and abiotic stress resistance in wheat breeding. However, a lack of domestication traits or regional adaptation to major bread wheat growing regions has hindered the use of this species in breeding for agronomic or end-use quality traits. To help address this gap, as well as to accelerate efforts to incorporate stress tolerance and disease resistance into elite wheat germplasm, we have developed a backcross introgression population, consisting of a diverse set of 25 wild emmer accessions crossed to a set of six elite hard winter wheat lines adapted to the US Central Great Plains. Here, we present an assessment of the length and distribution of introgression segments in this population, frequency of recombination events across the genome, and the frequency of aneuploid events in this interspecific cross, characterized using whole genome skim-sequencing. Additionally, we discuss early measurements of phenotypic diversity in this population, and identify several traits for which this population will contribute useful diversity into US wheat germplasm.

1929S **Optimizing expected cross value for genetic introgression- smart crossing design with whole-genome functional genomic knowledge** Pouya Ahadi[1], Balabhaskar Balasundaram[2], Juan Borrero[2], Charles Chen[3] [1]H. Milton Stewart School of Industrial and Systems Engineering, Georgia Institute of Technology, [2]School of Industrial Engineering and Management, Oklahoma State University, [3]Biochemistry and Molecular Biology, Oklahoma State University

In plant and animal breeding, the pursuit of superior phenotypes is essential to meet the breeding objectives of enhanced productivity, increased financial returns, improved welfare, and reduced environmental impact. Traditionally, these breeding goals are achieved by identifying the individuals with desirable traits and crossing them to create the segregation of phenotypes in a new generation that allows further selection for advancement.

In this study, we address the mate selection problem in the hybridization stage of a breeding pipeline, which constitutes the multi-objective breeding goal key to the performance of a variety development program. The solution framework we formulate here seeks to ensure that individuals with the most desirable genomic characteristics are selected to cross, allowing for maximizing the likelihood of the inheritance of desirable genetic materials to the progeny. Unlike approaches that use phenotypic values for parental selection and evaluate individuals separately, or a few genetic markers, we use a criterion that employs the genetic architecture of target traits, considering the segregation and combination of the genomic information of the pair of individuals.

We, here, introduce the expected cross value (ECV) criterion, which quantifies the expected number of desirable alleles for a gamete produced by two selected individuals of the base population, providing a comprehensive assessment of all genetic potential. We have developed an integer linear programming formulation for this parental selection problem using the ECV criterion. The formulation is capable of controlling the inbreeding level between selected parents. Furthermore, we extend the application in two directions: (i) simultaneous improvement of multiple target traits, and (ii) finding multi-parental solutions for designing crossing blocks. We evaluate and discuss the performance of the ECV criterion compared other selection approaches. Finally, we explore how the ECV criterion and the proposed integer linear programming techniques can be effectively applied to improve the efficiency of genetic introgression while maintaining genetic diversity within breeding programs.

1930V **Exploiting admixture in livestock to inform the genetic architecture of important traits** Pamela Wiener[1], Juliane Friedrich[1], Richard Bailey[2], Andrea Talenti[3], Mazdak Salavati[4], Félix Meutchieye[5], Keith Ballingall[6], Guillaume Salle[7], Olivier Hanotte[8,9], Emily Clark[1], James Prendergast[1] [1]Roslin Institute, [2]University of Łódź, [3]Oxford Nanopore Technologies, [4]Scotland's Rural College, [5]University of Dschang, [6]Moredun Research Institute, [7]INRA, [8]University of Nottingham, [9]International Livestock Research Institute

Admixture, in which gene flow between genetically separated populations leads to the production of individuals with complex ancestry, is an important process across the biological spectrum. Human-mediated admixture (hybridization, cross-breeding) has played an influential role in the history of domesticated animals, leading to the development of new breeds, local populations and commercial lines. Modern approaches in genotyping allow the genetic dissection of the impact of admixture on individual genomes, which can be exploited to address important issues in genetics and agriculture. This work, encompassing two studies, involves the application of population genetics techniques to admixed livestock populations in order to characterise ancestry across the genome and to identify loci of specific ancestry or with steep genomic clines (gradients in allele frequency across the overall range of admixture levels), providing evidence of association with restricted cross-breeding.

In one study, we used SNP array data to confirm previous results that some Caribbean sheep have West African and European origins, presumably due to introduction of sheep from both geographic regions. We then identified genomic regions with an excess of West African ancestry, which contain genes that are candidates for adaptation to tropical environments. In a separate study, we analyzed genotypes generated by whole-genome sequencing to characterize genomic clines in a number of African cattle populations, which vary in the level of admixture between taurine (Near Eastern origin) and indicine (South Asian origin) lineages. This analysis identified many genomic regions with steep clines, indicating restricted introgression, which may be related to genomic incompatibilities and reproductive isolation between taurine and indicine backgrounds. These studies provide insights into the genetic basis of environmental adaptation and hybrid incompatibility. In addition, they may contribute to breeding and management strategies in livestock, e.g. related to improved heat tolerance.

1931T **Genomic Evidence of Selection on Gall Size among Goldenrod Gall Fly *Eurosta solidaginis*** Zhenzhu Xiao[1], Arthur Weis[2], Linyi Zhang[1] [1]Department of Biological Sciences, George Washington University, [2]University of Toronto

Predicting the evolution of traits in response to natural selection is a fundamental pursuit in evolutionary biology. Achieving this goal necessitates a holistic approach, connecting genotype, phenotype, and fitness. However, such investigations are often limited, either due to the complexity of pinpointing the trait under selection or the scarcity of genomic resources for trait elucidation. In this study, we aim to bridge this knowledge gap by identifying the genomic signatures of natural selection acting on a pivotal phenotype: gall size in goldenrod gall flies—a classic model for studying multitrophic interactions in trait evolution. Previous research has established key insights: 1) gall size exhibits heritability among these insects, 2) small galls face elevated predation risk by parasitoid *Eurytoma gigantea*, and 3) large galls are more susceptible to avian predation. This ecological interplay generates stabilizing selection on gall sizes within populations. Our study's objective is to detect genomic evidence of selection acting on gall sizes. To achieve this, we collected gall samples from three distinct sites, encompassing the time points before any natural enemies' attacks, after parasitism events, and subsequent to bird predation. We conducted a genome-wide association study involving 736 individuals to uncover the genetic architecture underlying gall size variation. Furthermore, we employed pool sequencing across these three time points to track allele frequency changes and corresponding phenotypic scores in gall sizes over time. Our investigation seeks to discern whether allele frequency changes occur among loci responsible for gall size, shedding light on the genomic underpinnings of this key ecological trait's evolution in response to natural selection.

1932T **Repeatability of evolution varies across timescales in the introduced African Fig Fly, *Zaprionus indianus*** Jerry He[1], Alyssa Bangerter[2], Logan Rakes[1], Ansleigh Gunter[1], Alan Bergland[2], Priscilla Erickson[1] [1]University of Richmond, [2]University of Virginia

Invasive species are immensely successful in new environments, but the biological mechanisms that enable their spread remain elusive. Some invasive species show rapid adaptation to new habitats, but few studies have captured post-invasion adaptation in real time. The African Fig Fly, *Zaprionus indianus*, arrived in North America two decades ago and rapidly spread northwards along the East Coast. We have tracked *Z. indianus* populations over six years in two temperate Virginia orchards where they are locally extirpated each winter and re-colonize each spring from an unknown source. With a generation time of three weeks, these introduced populations potentially adapt for 5-6 generation in new environments. We hypothesized that populations undergo predictable post-colonization evolution across multiple locations and years, resulting in repeated changes in allele frequencies across time and space. Using a new chromosome-level genome assembly for *Z. indianus,* we have sequenced over 200 individual flies and 34 pooled samples to estimate allele frequencies over time in Virginia and across a 20° latitudinal transect in the eastern United States. While North American populations show little genome-wide population structure, we identified a haplotype on the X chromosome that is highly geographically differentiated across multiple years of sampling. Analysis of pooled samples recapitulates this finding and identifies a second latitudinally differentiated autosomal locus. In contrast to our expectations, we find unpredictable post-colonization evolution across replicate orchards and years. In one large Virginia orchard, we detect a modest signal of parallel post-colonization allele frequency changes across six years of sampling. However, the variants that change predictably in this orchard tend to change frequency in the opposite

direction in a second orchard approximately 100 km away. Founder effects result in early colonizing populations with different genetic compositions each year, but throughout the course of the growing season, the populations become similar to one another. In contrast, we find parallel long-term changes in allele frequencies in Virginia and Florida over several years of sampling, suggesting that North American populations are undergoing similar changes over longer time periods. Our ongoing studies show that rapid evolution in highly polymorphic invasive species may be common, but the repeatability varies across timescales and populations.

1933T **Characterizing seasonal changes in the microbiome of Apis mellifera** Bronwyn M Boyd, Clare Scott Chialvo Biology, Appalachian State University

Pollinators play a critical role in maintaining balanced ecosystems, and the service provided by these species is essential to both wild flowering and crop species. Despite their ecological importance, pollinator populations are declining worldwide due to anthropogenic change. One such pollinator, the western honey bee (*Apis mellifera*), is one of the primary species responsible for the pollination of food crops. Since 2006, scientists have been monitoring the health of honey bee populations due to significant losses resulting from a combination of various emergent and pre-existing environmental threats, such as varroa mites, climate change, and beekeeping practices such as queen trade. Given their importance to agriculture, there is a need to develop new mechanisms for assessing and improving hive health. In this study, we seek to quantify how the gut microbiome of worker bees changes over the course of a season and identify whether these changes correlate with phenotypic markers of hive health. We used Oxford Nanopore sequencing to characterize the microbiome of pooled worker bee samples collected from 20 hives located in Watauga County, NC at four time points. The results of our study will help to expand our understanding of how temporal factors influence changes in the microbiome and their association with the hive health.

1934T **CRISPR/Cas9-based homing gene drive for population control of the crop pest, *Drosophila suzukii*** Amarish K Yadav[1], Cole Butler[2], Akihiko Yamamoto[3], Alun L Lloyd[2], Maxwell J Scott[3] [1]Entomology and Plant pathology, NC State University, [2]Biomathematics Graduate Program and Department of Mathematics, NC State University, [3]Entomology and Plant Pathology, NC State University

CRISPR/Cas9-based homing gene drives for biased inheritance of genetic elements are currently being explored for the genetic suppression of human disease-vectors and agriculture pests. Genes required for female development, such as *doublesex* (*dsx*), have been identified as a potential target of gene-drives for pest population suppression. *doublesex* transcripts undergo sex-specific alternative-splicing to produce male and female Dsx isoforms required for proper sexual and somatic tissue differentiation. Here, we have established split homing drives which target the *dsx* gene in *Drosophila suzukii*, an invasive crop pest. The gene drive element, containing *dsx* single guide RNA and DsRed genes, was introduced into the coding region (CDS) of female-specific exon of *dsx*, which is essential for function in females but not males. However, in most strains, hemizygous females showed dominant sterility due to male *dsx* transcript production. A modified homing drive that included an optimal splice acceptor site to prevent the male splice site rescued the dominant sterility in females. Super-Mendelian inheritance of the gene-drive cassette (94-99%) were noted with a line that expressed Cas9 with two nuclear localization sequences (NLS) under the *D. suzukii nanos* promoter. Interestingly, mathematical modeling suggests that these strains could be used for suppression of lab cage populations of *D. suzukii* with repeated releases at relatively low release ratios (1:4). We are currently evaluating the potential of these homing strains for genetic suppression of lab cage populations of *D. suzukii*. In addition, with the aim of reducing the potential for resistance to develop, we have made new drive strains that express two or more gRNAs targeting *dsx*.

1935T **Tempo-spacial distribution of Drosophila** Chau-Ti Ting, Shun-Chern Tsaur National Taiwan University

Taiwan has a particular terrain, with altitudes ranging from lowland to mountains of 4,000 meters above sea level. The climate varies from subtropical, tropical, and temperate to frigid zones, coupled with abundant rainfall breeding a rich insect fauna. This study takes advantage of the unique condition to investigate Central Taiwan's altitudinal distribution and species abundance of drosophilid flies. We spent one week each in February 2020 and August 2023 on the Central Cross-Island Highway to conduct surveys from the lowland to 2,600 meters. Five traps were set up, each with a 400-meter altitude separation, supplemented by sweeping actions along the road and into the forest areas. In total, 4,466 and 380 fruit flies were collected, totaling 21 genera and 110 fruit flies. Preliminary analysis of fruit fly species in the mid-altitude (alt. around 1,000 meters) are more abundant in species diversity. This diversity provides a general basis for studying the species and genetic diversity.

1936T **Character evolution in the *Impatiens* genus (Balsaminaceae): a statistical approach** Sudhindra R Gadagkar, Nicholas J Beck Midwestern University

The plant family Balsaminaceae (Order Ericales) comprises only two genera, Impatiens and *Hydrocera*, which are very

dissimilar. While *Hydrocera* is monotypic with *H. triflora* as its only species, Impatiens ranks among the largest angiosperm genera, containing more than 1000 species that are diverse and taxonomically complex. For these reasons, this exploratory study used the Impatiens genus to study character evolution. Nucleotide sequences for three intragenic DNA spacers (ITS, atpB-rbcL, and trnL-F) of 150 species of *Impatiens* and three outgroup species were obtained from Yu et al. (2015) to infer the phylogeny using maximum likelihood. Forty-six morphological and morphometric traits, also obtained from the study were superimposed onto the tree to study their evolution. Instances of parallel and de novo evolution were found. Traits were correlated using the statistic Cramer's V after eliminating those that exhibited significant phylogenetic signal, determined using Pagel's λ. Categorical principal components analysis was used to reduce the dimensionality of the 46 traits to a few underlying meta traits. The results of this study are presented here.

1937F **Characterization of Triclocarban (TCC) activity in the *Drosophila melanogaster in vivo* model** Adriana Muñoz Hernández, David García Mendoza, Patricia Ramos Morales Biología Celular - Genética y Toxicología Ambiental-Banco de Moscas, Universidad Nacional Autónoma de México, Facultad de Ciencias

Triclocarban (3,4,4-trichlorocarbanilide, TTC) is a common ingredient in personal care products often used in combination with Triclosan (TCS). Since the COVID-19 pandemic, the use of multiple disinfection and cleaning products containing TCC has increased. Although it has been used for fifty years, it has recently been of concern since it has been pointed out that it could act as an endocrine disruptor that implied a significant hazard to ecosystems, both due to its extensive use in multiple cleaning and disinfection products, as well as its persistence and environmental distribution. TTC is among the top 10 Contaminants of Emerging Concern (CEC). Once products containing TCC are applied to the skin (or breathing) it enters the body where is metabolized by cytochrome P450 enzymes, and their metabolites constitute a potential risk to human health. In this work, the activity of TCC was evaluated in two wild type strains of *Drosophila melanogaster*: CS (regular metabolism) and ORR (modified metabolism) to determine its toxicity and the extent of the consequences of a unique exposure across several generations. Third instar larvae were fed for 48 h with TTC [1.78E-06 – 26.4 mM]. For each concentration, the number of treated flies (t, F0) recovered (Survival, S) and the Sex Ratio (PSx) were recorded. To evaluate the reprotoxic effect over generational time, each generation 15 pairs of flies, 1♀ t x 1♂ t from each concentration or from their respective negative control were put independently into vials with *Drosophila* food free of TCC. Once the adults (F1 to F3) were recovered, the number of females and males were register and morphological review was made using a SMZ-445 stereo zoom. The fertility, average of progeny per family (PPF) and sex proportion from treated flies (F0) or those from F1 to F3 flies was compared to that of their respective control flies. TCC clearly affected the CS flies, being lethal from 2.5 mM and, although it affected the average of progeny per family of the CS and ORR flies, adults were recovered for the later strain at the highest concentrations. The use of different biomarkers favors the analysis of the response obtained. To evaluate the effects over time of compounds of concern is of priority to know the real hazard that implied for life and the environment. Acknowledgement: PAPIIT (IN226923), DGAPA-UNAM. Keywords: Triclocarban, emerging contaminants, *Drosophila*, endocrine disruptor, reprotoxic. Topic: Ecological Genetics and Genomics: Impact of environmental damage on biodiversity.

1938F **Wolbachia Improved Fitness Traits of *Drosophila* Fed Rotten Fruit Diet** Oluwatobi E Fijabi[1,1], laura k Reed[2], Alex Wheelock[3], Maria Rico Lopez[4] [1]University of Alabama, [2]Biological Sciences, University of Alabama, [3]Bates College, Lewiston, [4]Saint Xavier University, Illinois

Host-microbe interaction is essential in the face of temperature shifts due to global climate change. *Wolbachia*'s influence on *Drosophila's* fitness traits is variable and contextual. Evidence of *Wolbachia*'s transition from parasitism to mutualism is known based on evidence of its provisioning of nutrients to its host. However, this evidence is limited to assessing its influence on lab diets different from *Drosophila's* encountered natural diet. We hypothesize that *Wolbachia* status will influence the fitness traits of *Drosophila* fed strawberries under acute stress. We expect a positive impact on fitness components driven by provisioning between *Wolbachia* and the host. We approached this question uniquely using three DGRP lines naturally infected with *Wolbachia* and their *Wolbachia*-cured counterparts. We eliminated maternal bacteria, then larvae emerged on rotten strawberries until they were sexed. They were exposed to cold stress (4° without food, 15min recovery at 25˚C), heat stress (38˚C for 45min without food, 3.5hrs recovery at 25˚C), or control condition (4hr 15min at 25°C without food). All flies further recovered on food for at least 12 following treatments before phenotyping. We measured fecundity, weight, longevity, climbing performance, emergence survival, and starvation resistance as a proxy for fitness. Our result revealed sexual dimorphism across phenotypes. *Wolbachia* status did not influence emergence survival. Male flies are better climbers. Wolbachia infection status interacted with sex, genotype, and thermal stress conditions to impact the phenotypes of starvation, climbing performance, fecundity, longevity, and weight of strawberry-fed flies at emergence. In sum, our results showed a positive impact of *Wolbachia* in combating environmental stress. This impact, however, is genotype, sex, and stressor dependent. This study is essential for understanding the ecological and seasonal adaptations of holobionts

1939F **Quantifying compositional variability in microbial communities** Maike L Morrison[1], Katherine S Xue[2], Noah A

Rosenberg[2] [1]Biology, Stanford University, [2]Stanford University

Microbial communities present new challenges for the development of statistics for diversity measurement. To understand the composition of microbial communities and the differences among communities across spatial locations, time points, or experimental replicates, we present a new normalized measure for characterizing variability across multiple microbiome samples. The statistic relies on the diversity partitioning framework employed by Fst, with samples playing the role of "populations" and taxa playing the role of "alleles" [1]. Its convenient mathematical properties--such as its commensurability across different numbers of taxonomic categories and different numbers of communities considered--allow users to compare its values between disparate data sets. Extensions incorporate phylogenetic similarity among taxa and spatial or temporal distances between communities. In a longitudinal analysis of gut microbiomes of healthy adults taking an antibiotic, we are able to both quantify the increase in temporal variability of microbiomes following the antibiotic course and to measure the duration of the antibiotic's influence on microbial variability.

[1] Morrison, M. L., Alcala, N., & Rosenberg, N. A. (2022). FSTruct: An FST-based tool for measuring ancestry variation in inference of population structure. Molecular Ecology Resources, doi.org/10.1111/1755-0998.13647.

1940F **CRISPR-Cas9 on Red Palm Weevils Yellow-e gene** Adilla Razali[1], Ligia Cota Vieira[2], Ling Li[3], Girlie Agbayani[3], Khaled Amiri[3], Claude Desplan[2] [1]CGSB, New York University Abu Dhabi, [2]New York University Abu Dhabi, [3]UAE University

The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) originating from tropical Asia is a harmful pest of more than 30 palm species, including economically relevant crops, such as date palms, coconut palms and oil palms. In the Middle East, date palms particularly are crucial, as they are the main cash crop in this region. The most harmful stage of this pest is the larval stage, where they destroy the trunk of the palm from inside and eventually killing it. Pesticides are the most common method used to control this pest, which elicits negative impacts on the environment. We are trying to develop a more sustainable and eco-friendly strategy to control the red palm weevil population by using genetic control- CRISPR/Cas9. CRISPR/Cas9 is a recognized gene editing method, that has been vastly used on a variety of species, but it has never been used on the red palm weevil. Our analyses show that we have successfully edited the *yellow-e* gene in the red palm weevil using this method. Our data also showed that the loss of this gene causes the changes in coloration, impacts pupa to adult transition and increased mortality due to dehydration.

1941F ***Steinernema* nematodes as an emerging genetic model to study microbial symbiosis** Mengyi Cao Carnegie Institution for Science

Nematodes are among the most abundant and diverse groups in the soil fauna and they play a significant role in soil ecology. The associations between the soil-dwelling entomopathogenic (EPN, insect-parasitic) nematodes of *Steinernema spp.* and their naturally occurring mutualistic bacteria in the genus *Xenorhabdus* have been established as experimental models and have great potential for agriculture due to their antagonistic effects on agricultural pests. The transmission stage of the *Steinernema* nematode is a developmentally arrested infective juvenile (IJ) that carries symbiotic bacteria in an intestinal pocket. The IJ invades an insect via a repertoire of host-seeking behaviors, releases the symbiont, and reproduces within the insect cadaver. The next-generation IJ progeny leaves the insect cadaver to seek for a new insect host. In this talk, I will present our recent success in the development of both forward and reverse genetics tools in the nematode host to increase the power of *Steinernema-Xenorhabdus* as a model system to study microbial symbiosis. I will also discuss the ongoing projects exploring the application of this symbiotic system in various directions.

1942F **Genome-wide analysis of mutation in cadmium exposure in adapted and non-adapted *Daphnia pulex* genotypes** Nathan Keith[1], Craig E Jackson[2], Stephen P Glaholt[1], Kim Young[3], Joseph R Shaw[3] [1]O'Neill School of Public and Environmental Affairs, Indiana University, [2]O'Neill School of Publican and Environmental Affairs, Indiana University, [3]Indiana University

Exposure to chemical pollutants can alter the rate, and genome-wide distribution of germline mutations. However, studies measuring the effect of chemical exposure on mutation rate and spectra have not considered the eco-evolutionary background of studied genotypes, which could potentially influence the rates and patterns of germline mutations upon chemical exposure. Utilizing *D. pulex*, we conducted a comprehensive experiment to test our overarching hypothesis that adaptation to chemical pollution also protects the germline from mutagenesis. We, 1) identified *Daphnia pulex* populations that have adapted to live in mining-devastated regions by increasing their tolerance to cadmium. 2) We completed a large-scale MA experiment with an adapted genotype to measure the rate of germline mutation in both control conditions and an environmentally relevant concentration of cadmium. 3) We compared these MA experiment results to a previously reported and identically designed MA experiment with a non-adapted genotype. We report that patterns of cadmium-induced mutagenesis in the adapted

genotype were reversed compared to our previous observations in a non-adapted genotype. Cadmium exposure altered the single nucleotide mutation (SNM) rate in the same genome regions in adapted and non-adapted genotypes, but the rates were changed in opposite directions. Cadmium also altered specific SNM classes (e.g., A:T > G:C) in these genotypes in opposite directions. Cadmium mutagenesis in the non-adapted genotype was linked to oxidative DNA damage and interference with DNA repair mechanisms via cadmium replacing zinc in zinc-containing protein domains. The reversal of germline mutational trends in the adapted genotype suggests protection against cadmium toxicity, and we demonstrate that adapted populations have elevated copy-number, and expression levels of metallothionein, the protein that protects against cadmium toxicity by binding to cadmium irreversibly.

1943F **Chloroplast Phylogenomics of the Genus *Morus L.* (Moraceae)** Bibek Adhikari[1], Sanam Parajuli[1,2], Madhav P Nepal[1] [1]Biology & Microbiology, South Dakota State University, [2]Biology and Microbiology, South Dakota State University

There are approximately 13 species of mulberries (Genus *Morus*; Family Moraceae) distributed across temperate to subtropical regions of the world. *Morus rubra* – Red mulberry, native to North America – is an endangered species found in riparian areas of eastern North America. The species integrity of *M. rubra* is threatened by introgressive hybridization with its invasive exotic congener *M. alba* (white mulberry) challenging the species identification. To address this challenge, and threats posed by hybridization to the species integrity of *M. rubra*, we analyzed chloroplast genomes of 45 specimens including *M. rubra*, *M. alba*, and hybrid individuals. The average *M. rubra* chloroplast genome size is 159,415 bp with 128 genes coding for 83 proteins, 8 rRNAs, and 37 tRNAs. The chloroplast genome of *M. rubra* is larger than that of *M. alba* by 122 bp. The DNA sequence polymorphism analysis segregated the 45 samples into 13 haplotypes – 12 belonging to *M.* rubra and a single *M. alba* type. The coding sequences were found to be conserved within and in between the species and the variation was observed mostly in the non-coding and intergenic spacer regions. The phylogenomic analysis of the chloroplast genomes of 11 species supported previously published *Morus* phylogeny with well-resolved Asian and North American clades. Nine hypervariable sites were identified across the *Morus* genomes, which are yet to be tested for their effectiveness as species-specific markers. The results from this study not only provide insight into the *M. rubra* chloroplast genomes structure but also provide a foundational data for addressing complex taxonomic issues, genus biogeography, and understanding population genetics and hybrid swamp due to introgressive hybridization.

1944S **Cracking the *Drosophila* Eggshell: Identifying Genes Essential for UVB Sensitivity** Lillian Pennington, Llewellyn Green, Erin S Kelleher The University of Houston

Throughout development, organisms are continuously exposed to environmental mutagens, such as ultraviolet (UV) light emitted from the sun. UV radiation poses a significant threat to terrestrial organisms, including Drosophila melanogaster. During embryogenesis, a pivotal stage in the Drosophila life cycle marked by rapid mitotic divisions, it is imperative to minimize DNA damage caused by UVB radiation for proper development. It's important to recognize that in Drosophila and other oviparous organisms, embryogenesis occurs in the external environment, leaving the embryo unprotected from UVB radiation. In response to UV-induced DNA damage, Drosophila express genes encoding specialized DNA repair enzymes called photolyases. While several DNA repair genes have been recognized for their role in protecting Drosophila from UVB irradiation, the requirement for specific eggshell components in UVB tolerance has not been investigated. As the outermost membrane of Drosophila eggs, the eggshell guards the developing embryo from external threats, including UVB radiation. Therefore, we anticipate that specific proteins within the eggshell play a vital role in protecting the developing egg against UVB radiation. To identify eggshell genes essential for UVB protection in Drosophila, we are analyzing the UV sensitivity of a panel of eggshell mutants. The lethality of the eggshell mutants will be compared with that of DNA repair mutants to assess the importance of various eggshell genes in UVB protection. Several mutants have shown similar mortality to DNA repair mutants when exposed to UV radiation, revealing the role of certain eggshell proteins in UV irradiation sensitivity. Identifying UV-tolerant eggshell genes in Drosophila is significant because it can enhance our understanding of how oviparous organisms protect themselves from UVB rays.

1945S **Conservation of the Regulatory Region and the Genes they Regulate within a Network** Chinmay P. Rele, Laura Katie Reed Biological Sciences, The University of Alabama

Understanding how protein-coding genes are regulated paints a clearer picture of how the proteome and genome interact with the environment and each other. Regulatory regions are important for the temporal expression patterns of genes. The sequences of regulatory regions of genes are highly variable, both across species and within species. We are studying the evolution of protein-coding sequences and the regulatory regions of genes within the Insulin-signaling pathway (INS/Tor) and how their evolution correlates with other characteristics of the genes within the context of a network. Insulin-signaling genes are interesting within Drosophila because they include duplications within the genus and variation in the number of genetic and physical interactions. In this study, we use computationally-generated predictions from the RefSeq pipeline to annotate the genome, with special interest on the 63 genes assigned to the INS/Tor pathway in 32 Drosophila species.

These same genes and species are also being used by the Genomics Education Partnership to establish high-quality hand-curated gene annotations that will later be used to improve the predictions of the analysis represented here. The conserved elements identified within regulatory regions upstream of genes are likely cis-regulatory elements of those genes. We predict that conservation in these upstream regions are correlated with metrics of the gene such as dN/dS, intron size, indel abundance, number of interactions with other genes and proteins, and metrics describing network topology. We predict that the conservation of the regulatory regions and the CDS are positively correlated. We also predict that the conservation of the regulatory regions/genes are correlated with centrality measures such as eigenvector centrality, and also with their "hubness" – highly connected genes connected to other highly connected genes are more conserved. Regulatory regions of genes with shortest average path length to all the other genes in the network are also predicted to be the most conserved. We believed this would imply that the conservation of a gene is affected by its direct interactors, but the conservation of its regulatory regions is more affected by how much its associated gene connects to all other genes in the network. Understanding the interplay between network topology and conservation of the regulatory region and CDS will improve our understanding of how the genome and interactome evolve over time.

1946S **Diapause experience has lasting effects on post-diapause adult brain gene expression in monarch butterflies** Samuel M Stratton[1], Delbert A Green[2] [1]EEB, University of Michigan, [2]University of Michigan

Diapause is an induced state that enables organisms to halt development, becoming longer-lived and more stress-tolerant, in order to withstand environmentally harsh conditions. Then after a biologically determined amount of time and environmental conditions become favorable, development is resumed. While the dynamics of diapause, from entry to exit, are becoming well understood at the genetic and molecular level, it is less clear what are the lasting consequences of the diapause process once development is resumed. The monarch butterfly provides a captivating system to address this question as monarchs enter reproductive diapause in the fall which coincides with their migration from northern USA and southern Canada to central Mexico. This occurs in order to ensure sufficient resource allocation for migratory success. Here, two groups of monarch butterflies were reared under different pre-adult conditions, either natural fall (diapause inducing) or laboratory summer (direct development). Then these groups were raised in identical natural fall conditions as adults. After outdoor entrainment, individuals were then brought indoors to a fall-like incubator to further standardize experience before sequencing. We find that that fall conditions during pre-adult development were necessary to induce diapause. Further, among the group with pre-adult fall rearing, because the fall-like incubator was slightly above diapause-breaking temperatures, there was heterogeneity in individual reproductive maturation. RNA-seq analyses on the brains of these individuals uncovered that post-diapause, reproductively mature individuals retained signatures of diapause such as upregulation of genes related to DNA repair, DNA maintenance, maintaining tissue integrity, along with downregulation of genes related to cell division. Ultimately, these results showcase the lasting effects of diapause and encourage further questioning on the ecological and evolutionary consequences of this phenomena.

1947S **Investigating the non-homologous end joining (NHEJ) and single-strand annealing (SSA) pathways of DNA double-strand break (DSB) repair with single-allele resolution after CRISPR/Cas9 treatment in embryos of the Major Dengue Vector, *Aedes aegypti*** Joseph S Romanowski[1,2], Hitoshi Tsujimoto[1], Kevin M Myles[1], Zachary N Adelman[1] [1]Entomology, Texas A&M University, [2]Graduate Program in Genetics and Genomics, Texas A&M University

The *Aedes aegypti* mosquito is a major human disease vector estimated to be responsible for over 400 million dengue virus infections each year and is the focus of genetic control approaches. Tools such as CRISPR/Cas9 induce DNA double-strand breaks (DSB) which can achieve desirable, DNA repair-mediated edits for these control strategies. The repair mechanisms that drive these sequence changes, however, are not well understood. Here, we report an *Aedes aegypti* embryo assay capable of studying the non-homologous end joining (NHEJ) and single-strand annealing (SSA) pathways of DSB repair with genomic DNA ready for sequencing in as little as 24-hours post-CRISPR/Cas9 micro-injection. Paired with polymerase chain reaction (PCR) and Oxford Nanopore sequencing technologies, this assay allows for both single and pooled embryo data with single-allele resolution of DNA repair events, providing information about how factors such as DSB site can influence the NHEJ and SSA repair pathways to inform self-eliminating gene drive control approaches.

1948S **Using DNA metabarcoding to evaluate the diet of sea lamprey (*Petromyzon marinus*) in the Great Lakes** Conor O›Kane[1], John Robinson[2], Kim Scribner[2], Weiming Li[2], Jeannette Kanefsky[2], Nicholas Johnson[3], Tyler Bruning[3] [1]Fisheries & Wildlife, Michigan State University, [2]Michigan State University, [3]United States Geological Survey, Hammond Bay Biological Station

The invasive sea lamprey (*Petromyzon marinus*) is a harmful hematophagous ectoparasite of Great Lakes fishes that requires international control. Current annual assessments of sea lamprey control program success include the observation of wounds on native lake trout (*Salvelinus namaycush*) to estimate sea lamprey damage and extrapolate mortality rates. However, due

to the difficulty of obtaining a representative sample of Great Lakes fishes and challenges involved with the assessment of blood diets, comprehensive dietary analyses for sea lamprey are lacking. Previous studies using stable isotope and fatty acid analyses indicate sea lamprey diet composition varies within and across the Great Lakes, but these methods cannot reliably identify individual species in sea lamprey diets. With the advent of high-throughput sequencing technologies and improved accuracy of species-level identification, DNA metabarcoding may allow for a more extensive examination of sea lamprey diets given the presence of DNA in fish blood cells. The purpose of this research is to use molecular investigations of dietary composition to address three objectives: 1) identify an effective blocking primer to suppress sea lamprey DNA amplification 2) characterize retention times of observable DNA under experimental conditions within sea lamprey digestive tracts at different temperatures and fasting periods, 3) compare the detectability and composition of host DNA after consecutive feedings on multiple species. We developed and tested eight blocking primers with varying base pair length, end modifications (C3 spacer and inverted dT), and purification methods via gel visualization, quantitative PCR, and DNA metabarcoding. Between samples the average Ct value difference with and without blocking primers was 13.72 (sd = 0.95), indicating a 13,520x average suppression of sea lamprey DNA amplification. We then evaluated retention times of dietary DNA in sea lamprey across five fasting periods (0, 5, 10, 20, 30 days), following a seven-day feeding period at three temperatures (0, 5, 10 °C). Following metabarcoding and subsequent bioinformatic analyses, sequence read counts were compared to investigate a relationship between fasting period length and temperature on dietary DNA detectability. Additionally, similar relationships were explored from sea lamprey allowed consecutive feedings on two different species to assess detectability of both hosts

1949S **Environmental DNA metabarcoding reveals community-wide patterns of aquatic invertebrate gene flow and genetic drift in Grand Canyon.** Jared Freedman[1], Theodore A Kennedy[2], Molly K Burke[1], David A Lytle[1] [1]Integrative Biology, Oregon State University, [2]Grand Canyon Monitoring and Research Center, USGS

The spatial distribution of genetic diversity in river networks reflects complex influences of landscape characteristics on the evolutionary and demographic histories of aquatic organisms. Aquatic invertebrates with complex life histories face unique barriers to gene flow, such as stream network configuration, inhospitable terrestrial landscapes, and anthropogenic habitat alterations, that influence community-wide patterns of genetic connectivity. However, widespread collection of genetic samples from multiple species of interest is often prohibitively difficult. To detect community-wide genetic patterns, we used environmental DNA (eDNA) metabarcoding to simultaneously capture community and population genetic data for aquatic invertebrates in the Grand Canyon reach of the Colorado River. Trace DNA was obtained from water samples at 36 sites in the Colorado River and its perennial tributaries, which was then sequenced using a degenerate primer set that targets a fragment of the aquatic invertebrate COI gene. We utilized synthetic spike-in PCR standards of known copy number to obtain normalized between-sample quantification of each species, as well as strict denoising and filtering parameters to extract haplotype level information for many aquatic invertebrate species. Pairwise measures of genetic distance and river network distance were compared to assess the regional influence of gene flow and genetic drift across the Grand Canyon ecosystem. In total, we identified 1,201 haplotypes across 448 species, with 80 species containing sufficient genetic variability to perform population genetic analyses. 55 of these species exhibited low regional gene flow, 11 exhibited moderate regional gene flow, and 8 exhibited high regional gene flow. Further work is ongoing to incorporate more complex analyses that take into account multiple modes of ecological distances, including least-cost terrestrial distance and non-geographic environmental distance. These result suggests a widespread limitation to aquatic invertebrate gene flow between aquatic habitats in the Grand Canyon, particularly between geographically isolated tributaries. The ability of eDNA metabarcoding data to detect ecologically-relevant patterns of genetic variability presents a promising step forward for ecological genetics, as it allows for investigations of the impact landscape features and environmental gradients across entire communities.

1950S **Comparative genomics identifies adaptive immune genes experiencing differential selection in Hawaiian honeycreepers with potential applications for conservation.** John H. Neddermeyer[1], Michael G. Campana[2], Marc Tollis[3], Robert C. Fleischer[2], Jeffrey T. Foster[1] [1]Biology, Northern Arizona University, [2]Center for Conservation Genomics, Smithsonian National Zoo and Conservation Biology Institute, [3]Engineering, Informatics, and Applied Sciences, Northern Arizona University

Infectious disease has been one of the most constant selective pressures in the evolution of birds and mammals. Host genetics are frequently identified as a major component of infection outcomes in both humans and wildlife. Wildlife around the globe are imperiled by the introduction of novel infectious diseases, with devastating consequences for biodiversity, and the introduction of avian malaria to the Hawaiian Islands is a preeminent example. Avian malaria has been identified as a main driver of honeycreeper extinctions. The severe mortality observed in Hawaiian honeycreepers is likely due to an evolutionary history devoid of malaria causing parasites. The severe impact of the parasite on native bird species makes avian malaria in Hawai'i a unique model system for understanding selective pressures of disease. Using whole genome sequencing data from 17 extant and one recently declared extinct honeycreeper species we sought to use comparative genomic methods to identify genes contributing to disease susceptibility in Hawaiian honeycreepers. We assembled honeycreeper genomes from Illumina sequences using two approaches, de novo with 10X Genomics linked-reads and reference-guided using one of

the honeycreeper de novo assembled genomes as reference. Post assembly we assessed genome continuity and annotation completeness. A total of 41 genomes were included in analyses, 18 honeycreepers and 23 non-honeycreeper genomes downloaded from NCBI. Given that avian malaria is ubiquitous throughout the world and does not cause severe population declines a key assumption of our work is that honeycreepers are uniquely susceptible to avian malarial disease. Using highly conserved genes identified by BUSCO we constructed a species tree based on 7,592 gene trees to serve as input into Progressive Cactus. From our multispecies alignment, using the chicken genome as a reference, we identified adaptive immune genes for selection analyses. We determined if genes were under selection in honeycreepers, using branch-site methods implemented in HyPhy, and using PhyloP in PHAST. We found several adaptive immune genes experiencing differential selection in honeycreepers. Specifically, genes involved in MHC Class I and II signaling. Further, using codon-site selection tests we identified codons in pathogen recognizing motifs under differential selection in honeycreeper species. These analyses offer information at the scale of a single genome per species, but evolution often occurs at the population level. Genes involved in the adaptive immune response identified as experiencing differential selection in honeycreepers offer targets for population level studies, where allelic associations between honeycreeper infection and disease survival can be made.

1951S **Gene copy number promotes the rapid evolution of pests across Diptera** Dylan Padilla, Michael Angilletta School of Life Sciences, Arizona State University

Diptera is one of the most species-rich and ecologically innovative groups of organisms, making up 10-15% of all animal species. Historically, they have constituted a major group of pests that inflict serious damages to plants and animals, both directly by ingesting phloem/blood and indirectly as vectors of numerous diseases. Deciphering the evolutionary mechanisms responsible for the rapid evolution of pests and invasive species could enable us to apply control measures, preventing major ecological and even economic impacts. Here, we investigate the evolution of the *foraging* gene (*for*) across diptera; a gene known to alter the foraging behavior of certain species of flies. Through a gene-tree species-tree reconciliation analysis, we found multiple events of gene duplication, and gene loss throughout the history of dipterans. Interestingly, the number of *for* paralogs among species seems to be associated with the rapid evolution of pests. Our results show evidence of a potential mechanism underlying the rapid evolution of pests across dipterans, enabling us to mitigate relevant ecological and economic impacts of human concern.

1952T **Comparative Single-Cell Transcriptomic Analysis in C. elegans and C. briggsae Embryos** Rupa Khanal[1], Christopher R. Large[1], LaDeana Hillier[2], Chau Huynh[2], Priya Sivaramakrishnan[1], Felicia Peng[1], Qin Zhu[3], Erik Nordgren[1], Jean Rosario[1], Junhyong Kim[1], Robert H Waterston[2], John I. Murray[1] [1]University of Pennsylvania, [2]University of Washington, [3]University of California, San Francisco

Understanding the intricate mechanisms of gene regulation during the development of multicellular organisms is pivotal in evolutionary biology. C. elegans has a fully mapped invariant lineage, and it is nearly perfectly conserved in related species such as C. briggsae. This offers a unique opportunity to identify homologous cell states across the organism and therefore, compare transcriptomic dynamics between species at single-cell resolution.

We analyzed single-cell RNA-seq datasets comprising over 193,020 cells from C. briggsae embryos and 255,027 cells from C. elegans embryos. Through joint annotation and quantitative comparisons, we show that most well-expressed orthologous genes have a high conservation of their expression profiles between the two species. We classified genes based on expression breadth and conservation, and identified constraints including that broadly expressed genes are more likely than specifically expressed genes to have conserved expression. Genes annotated with certain functional categories such as transcription factors tend to have high conservation, while others such as genes annotated with neuronal function show more differences between species in embryonic expression.

By measuring transcriptome conservation of terminal and progenitor cell types across species, we uncovered several patterns. Both species exhibited a higher proportion of species-specific marker genes in early progenitor cells compared to later stages. In terminal cells, germline, muscle and intestine have more similar transcriptomes across species than most neurons and glia. Most hypodermal cell types have strong conservation of 1:1 ortholog expression, but also express more genes that have more complex orthology relationships. Examining transcriptome similarity across time showed that while on average lineages have a maximum similarity at the 200-350 cell stages, individual trajectories can deviate from this pattern.

In conclusion, our comparative analysis highlights the interplay of conservation and divergence in gene expression during embryonic development.This contribution deepens our insights into developmental biology and evolution on a broader scale.

1953T **Investigating the evolution of new body parts in the rapidly evolution genitalia of *Drosophila*** Gavin Rice[1], Tatiana Gaitan[2], Kenechukwu Charles-Obi[1], Julia Zeitlinger[2], Mark Rebeiz[1] [1]Department of Biology, University of Pittsburgh, [2]Stowers Institute for Medical Research

Recently evolved traits i.e., novelties, often represent key features that allow animals to exploit new ecological niches (e.g. feathers in birds) and can even help them find a mate (e.g. bioluminescence in fireflies). Yet few, if any, morphological traits we term as novel are entirely independent of other traits found throughout the body plan. Many novel traits have been hypothesized to be formed through genetic network co-option, where a genetic network becomes activated in a new part of the body plan. Yet even though this theory has been implicated countless times, few cases establish the sufficiency of network co-option to induce a novel morphology. Once a genetic network is deployed, how these networks become rewired to produce the trait's unique characteristics remains unclear.

The rapidly evolving genitalia of Drosophila provide a powerful system to study the developmental basis of morphological novelty. We investigated how the phallus of *Drosophila eugracilis* gained over 150 novel projections. Developmental tracking of cellular morphology uncovered evidence that these projections are unicellular. Larval trichomes, which have a well-characterized genetic network, are also formed as unicellular projections but are much smaller and differ in shape. Our analysis shows that the main transcription factor for the larval trichome network, Shavenbaby, and 14 of its known downstream targets have gained expression in the *Drosophila eugracilis* phallus. This suggests that the co-option of this network underlies this dramatic phenotype. In fact, activation of the larval trichome genetic network in the phallus of *Drosophila melanogaster*, which naturally lacks these unicellular projections, induces a partial phenocopy of the novel unicellular projection found in *Drosophila eugracilis*. This indicates that genetic network co-option is sufficient to induce novelties. Additionally, we find a portion of the *shavenbaby* genetic network differs when it is activated ectopically in *Drosophila melanogaster* with respect to the native projections of *Drosophila eugracilis*, highlighting nodes in the network that were likely added after these projections first evolved.

1954T **Understanding cellular and molecular developmental mechanisms of ovariole number determination across Hawaiian *Drosophila*** Cassandra Extavour[1,2,3], Adriana Aguilar-Maldonado[3] [1]Organismic & Evolutionary Biology, Harvard University, [2]Howard Hughes Medical Institute, [3]Molecular & Cellular Biology, Harvard University

Female reproductive fitness in *Drosophila* is highly heritable and determined by the egg-producing subunits of the ovary, the ovarioles. Previous work in our lab has characterized genetic, molecular, and cellular mechanisms underlying ovariole number variation in some *Drosophila* species, including *Drosophila melanogaster* and *D. sechellia*. However, we do not yet have a good understanding of which developmental mechanisms could be targets of evolutionary change for ovariole number determination. We have chosen two unique study systems to investigate the developmental and molecular mechanisms regulating ovariole number; the drosophilids of the *virilis* subgroup and those endemic of the Hawaiian Islands. These groups contain closely related flies that have diverged over relatively short time scales but that have evolved very different ovariole numbers as well as distinct ecological niches. I will discuss my progress in characterizing ovariole development and determining the cell biological basis of ovariole number determination in Hawaiian *Drosophila*, (2) investigate whether key genetic regulators within the mTOR, JAK/STAT, EGFR, or Hippo signaling pathways have been the targets of evolutionary change, and (3) determine the evolutionary changes in gene regulatory networks underlying ovariole number in the Hawaiian *Drosophila*. The ultimate goal of my work is to understand the developmental and genetic basis of adaptive evolutionary changes.

1955T **Characterization of Zebrafish Homologs of human P-gp and ABCG2 multidrug efflux transporters** Joanna Thomas, Robert Robey, William Frye, Paula Salazar, Collin Inglut, John Quinlan, Suresh Ambudkar, Andrew Warner, Donna Butcher, Jennifer Matta, Elijah Edmondson, Michael Gottesman National Institutes of Health

The ATP binding cassette (ABC) transporters P-gp (P-glycoprotein, encoded by *ABCB1*) and ABCG2 (*ABCG2*) were identified due to their ability to confer resistance to multiple different cytotoxic drugs in cancer cells. P-gp and ABCG2 are two of the most important multidrug efflux transporters, due to their wide and overlapping substrate specificities and diverse expression patterns. P-gp and ABCG2 are primarily expressed in excretory organs (e.g., liver, kidney, intestines) and at barriers to protected sites (e.g., blood-brain and placental barriers), and are therefore key drivers of substrate drug pharmacokinetics and distribution. Zebrafish are an ideal model to study drug toxicity and tissue distribution, but these efforts require a detailed characterization comparing the zebrafish and human homologs for translational relevance.

Previously, we characterized the 2 zebrafish homologs of P-gp (Abcb4, Abcb5) and found both had similar tissue expression patterns to human P-gp, but Abcb4 was the only homolog at the blood-brain barrier (BBB). A high-throughput analysis of substrate transport demonstrated a high correlation between Abcb4 and P-gp (R=0.94) with near perfect substrate overlap. Abcb5 shared fewer substrates (r=0.67) and is expressed at high levels in the zebrafish gills, skin and ovarian follicles.

Currently, we are characterizing the 4 zebrafish ABCG2 homologs (Abcg2a, -b, -c, -d). *abcg2a, -b* and *-d* shared similar expression patterns to human ABCG2, with all being detected in the liver, kidney, intestines and ovarian follicles. *abcg2c* was

only detected in the kidney and ovarian follicles. *abcg2a* was most highly expressed in the liver and intestine and was the only paralog at the BBB. *abcg2a* and *abcg2d* were the only paralogs expressed in the gills. Abcg2a had the greatest substrate overlap with ABCG2 whereas Abcg2d shared the fewest. This result was surprising as Abcg2d has the second highest amino acid identity with ABCG2. Computational modelling of predicted structures of Abcg2a-d aligned to ABCG2 demonstrated Abcg2d has a more divergent substrate binding pocket than Abcg2a, which may affect substrate recognition or transport.

In conclusion, we have identified Abcb4 and Abcg2a as the most phenotypically similar zebrafish homologs of P-gp and ABCG2, respectively. The similarity of substrate profiles and tissue distribution lends credence to the use of zebrafish for drug toxicology and PK/PD studies. Expression of these conserved transporters at the BBB paves the way for the use of zebrafish to study methods of increasing drug delivery to the brain.

1956T **_Blimp1_ is a pair-rule gene in the hemipteran _Oncopeltus fasciatus_** Leslie Pick, Katie Reding, Abigail Heath University of Maryland, College Park

Subdivision of the *Drosophila melanogaster* embryo occurs through a series of transcription factors that progressively define smaller regions of the antero-posterior (AP) axis. Among these are the pair-rule factors which specify pairs of parasegments along the axis and are encoded by the pair-rule genes (PRGs), which are expressed in alternating segment primordia. Mutation of any of these genes results in loss of every other segment - the classic pair-rule phenotype. First identified through *Drosophila* mutant screens in the 1970s-80s, the functions of the *Drosophila* PRG orthologs have since been studied in other insect species. Interestingly, in the milkweed bug *Oncopeltus fasciatus* (Hemiptera), knockdown of the *Drosophila* PRGs did not result in clear pair-rule phenotypes (1-3), while knockdown of the nuclear receptor-encoding gene *E75A* yielded embryos displaying loss of alternate segments (4). These results suggest that segmentation in *Oncopeltus* likely occurs according to a 'pair rule', but involves a different set of transcription factors from those deployed in the *Drosophila* embryo. In order to identify transcription factor-encoding genes involved in segmentation in the *Oncopeltus* embryo, we sequenced RNA from three time points during *Oncopeltus* embryogenesis. We selected transcription factor-encoding genes with similar transcriptional profiles as *E75A* to screen by in situ hybridization. We have so far identified one gene, *Of-Blimp1,* that is expressed in alternating segment primordia through much of germband elongation, suggesting possible pair-rule function. We used CRISPR/Cas9 to generate germline mutants and isolated five loss-of-function alleles which we have used to characterize the *Of-Blimp1* mutant phenotype. *Hox* gene expression patterns in *Of-Blimp1* homozygous embryos suggest that alternate segments are lost across much of the body axis. Mosaic G0 somatic mutants display fusions of pairs of segments at a register consistent with the patterns of segment loss observed in the germline mutants. To assess whether *Dmel-Blimp1* plays a role in segmentation, we also generated *Dmel-Blimp1* mutant lines and found that *Dmel-Blimp1* homozygotes do not display any defects in segmentation. These results suggest that while segmenting the AP axis according to a 'pair rule' may be ancestral to much of Insecta, the set of genes required for this phenomenon is more evolutionarily labile than previously thought.

1. Liu PZ and Kaufman TC (2005) *Development 132*(9): 2081-92.

2. Auman T and Chipman AD (2018) *BMC Evol Biol. 18*(1): 178.

3. Reding K, Chen M, Lu Y, Cheatle Jarvela AM, and Pick L (2019) *Development 146*(17).

4. Erezyilmaz DF, Kelstrup HC, and Riddiford LM (2009) *Dev Biol. 334*(1):300-10.

1957T **The _PAX_ genes of _Vanessa cardui_, the painted lady butterfly** Ximena Gutierrez-Ramos, Leslie Pick Entomology, University of Maryland

During embryogenesis, genes often work in networks related to a specific developmental process, for example segmentation or eye development. One family of genes that is important in both of these processes is the *Pax* family. The *Pax* genes are conserved across animals. These genes encode conserved transcription factors that share a domain that binds DNA in a sequence-specific fashion, the Paired domain, as well as additional domains that are restricted to specific family members (Blake & Ziman, 2014). *paired* (*prd*), the first described *Pax* gene, is required for embryonic development, functioning as a typical pair-rule gene in the fly *Drosophila melanogaster* (*Dme*). *Dme-prd* is an essential gene for development of alternate body segments, and in *prd*'s absence the embryo is unviable (Kilchherr et al., 1986; Frigerio et al., 1986). In recent work we demonstrated that in malaria mosquito, *Anopheles stephensi*, *prd* was lost and functionally replaced by another *Pax* gene, *gooseberry* (*gsb*) (Cheatle Jarvela et al., 2020). In a phylogenetic analysis we observed that *prd* was independently lost in some Lepidoptera genomes. To determine when during insect evolution *prd* was lost we performed a bioinformatic analysis to identify the Paired domain in 160 lepidopteran genomes. We found that *prd* is absent in all of the species examined, suggesting that *prd* was lost early in the radiation of Lepidoptera. We are using *Vanessa cardui*, the painted lady butterfly, as a model system to carry out functional experiments to understand the mechanism underlying *prd* loss.

Was *prd* replaced by *gsb* in *Vanessa*, as it was in *Anopheles*? Did another *Pax* gene replace *prd* or does a different mechanism explain the loss of a critical regulatory gene? We identified 10 *Pax* genes in the *Vanessa* genome, including *gsb*. We performed an *in situ* hybridization to determine the expression pattern of *gsb*, and at the moment we are doing knock down experiments with RNA interference to determine if *gsb* is functionally substituting for *prd* in the formation of alternate segments in the butterfly. With this work we are examining how gene networks can withstand loss of genes suffer alterations in their gene components and how they can rearrange without affecting important embryogenesis processes.

1958T **An lncRNA master switch for pigmentation and adaptive color variation in butterflies** Luca Livraghi[1,2], Joseph J Hanly[1], Chris D Jiggins[2], Arnaud Martin[1] [1]Department of Biological Sciences, The George Washington University, [2]University of Cambridge

Evolutionary variations in the wing pigmentation of butterflies and moths offer striking examples of adaptation by crypsis and mimicry. The *cortex* locus has been independently mapped as the locus controlling colour polymorphisms in 14 lepidopteran species, suggesting it acts as a genomic hotspot for the diversification of wing patterns, but protein-coding knockouts with CRISPR-cas9 have proven to be difficult to obtain. In this study, we unveil the role of a novel long non-coding RNA (lncRNA) called *ivory*, transcribed from the *cortex* locus, in modulating colour patterning in five nymphalid butterfly species. Strikingly, *ivory* expression prefigures most melanic patterns during pupal development. CRISPR mutants of *ivory* showed transformation of dark pigmented scales into white or light-coloured scales, while G_1 cortex null mutants had no phenotype, showing that *ivory* and not *cortex* is essential for normal pigmentation in butterflies. We suggest that G_0 crispant phenotypes for *cortex* could be explained by large deletions that perturbed non-coding elements. Overall, these results show that an lncRNA, rather than the *cortex* protein-coding gene, acts as a master switch of colour pattern specification that played key roles in the adaptive diversification of colour patterns across Lepidoptera. These findings highlight the significant influence of lncRNAs in developmental regulation, and also underscore their potential as key genetic players in the evolutionary process itself.

1959F **Female-limited color dimorphism in the *Drosophila montium* species subgroup as a model to understand the molecular mechanisms of sex-limited polymorphism and evolution of dominance** Yuichi Fukutomi[1,2], Emily K Delaney[1], Jingqi Liu[1], Olga Barmina[1], Masayoshi Watada[2], Seema Ramniwas[3], Artyom Kopp[1] [1]UC Davis, [2]Tokyo Metropolitan University, [3]Chandigarh University

Many animal taxa have evolved discrete phenotypic polymorphisms where each of the alternative phenotypes is adaptive in one sex and disadvantageous to the other. Theory predicts that the resulting intersexual conflicts can be resolved by the evolution of sex-limited polymorphisms or sex-specific dominance relationships between alternative alleles. Both phenomena have been observed in nature, but the developmental mechanisms that explain them are unknown. Abdominal pigmentation in the *Drosophila montium* species subgroup provides an excellent model for investigating both mechanisms. Many species in this clade display a Mendelian, female-limited color dimorphism (FLCD). GWAS analyses reveal that independent regulatory mutations in the POU domain motif 3 (*pdm3*) gene, a repressor of pigmentation, are responsible for the repeated evolution of FLCD in multiple species. Antibody staining shows that Pdm3 is expressed in both dark and light morphs, and in both males and females, suggesting that subtle quantitative differences in *pdm3* expression may be responsible for FLCD. At the same time, species with FLCD differ in the dominance relationships between dark and light *pdm3* alleles. While dark alleles are dominant in most species, there are exceptions such as *D. bocqueti*. We show that in *D. bocqueti*, the light allele is fully dominant at high temperatures, the dark allele is fully dominant at low temperatures, and the two alleles are co-dominant at intermediate temperatures. Our results are consistent with a hypothesis that abdominal pigmentation in the *montium* clade is a threshold trait, where continuous variation in gene expression is converted into discrete, Mendelian adult phenotypes. To test this model, we will quantify the expression of light and dark *pdm3* alleles in Light/Dark heterozygotes in different species and at different temperatures, and test (1) whether the two alleles cause quantitative differences in *pdm3* expression that explain their adult phenotypes, and (2) whether the expression levels of *pdm3* from light and dark alleles have distinct reaction norms that may explain temperature-dependent dominance in *D. bocqueti* and temperature-independent dominance in other *montium* group species.

1960F **The spatiotemporal evolution of *dumpy* regulation in the rapidly diversifying *Drosophila* genitalia** Catarina Colmatti Bromatti[1], Donya Shodja[2], Ben Vincent[2], Mark Rebeiz[1] [1]Biological Sciences, University of Pittsburgh, [2]University of Pittsburgh

The evolution of complex new structures, so-called “morphological novelties”, is a topic of intense study in the field of evolutionary developmental biology. Gene regulatory networks that control developmental expression are critical to explaining how new structures deploy genes to achieve their unique morphological features. In particular, we need examples that molecularly explain how genes become deployed in new tissues that can contribute to the emergence of a morphological novelty. Here, I use *Drosophila melanogaster* as a model organism to study a recently evolved novel genital structure called the

posterior lobe. Previous work has found that the posterior lobe derived an enhanced accumulation of the apical extracellular matrix (aECM) during its developmental evolution. The terminal effector Dumpy is present in the aECM, and disruption of *dumpy* causes dramatic the posterior lobe defects. In investigating how *dumpy* gained posterior lobe-associated regulation, I have identified multiple enhancers sufficient to drive reporter expression in and near cells of the posterior lobe, as well as elements that drive expression in the claspers and phallus, two other structures where endogenous *dumpy* is expressed in the developing genitalia. This suggests that these enhancers might be pleiotropic. My work suggests that multiple elements underlie the evolution of posterior lobe-associated *dumpy*, providing a much-needed window into how key terminal genes are co-opted in morphological novelties.

1961F **Comparative analysis of *Drosophila* seminal fluid investment: Trade-offs and evolutionary diversification** Dylan J Sims-West[1], Zeeshan Syed[1], Roberto A. Gomez[1], Patrick O'Grady[2], Scott Pitnick[1], Stephen Dorus[1] [1]Biology, Syracuse University, [2]Entomology, Cornell University

Seminal fluid proteins (SFPs) play key roles in reproduction and evolve rapidly. In *Drosophila,* SFPs are produced by the accessory glands (AGs), transferred to females during mating and influence a diverse repertoire of female postmating phenotypes. These effects can be profound and include female remating, sperm storage and use, ovulation and oviposition. Despite their functional importance, relatively little work has been done to examine their evolution in relation to PCSS within a phylogenetic framework. The *Drosophila* genus provides a powerful model for comparative investigations into the evolution of SFP investment, composition and function given the availability of genomic resources and the recent completion of the *Drosophila* Evolutionary Phenomics (DEP) resource. Here, we report preliminary results from an investigation of the impact of PCSS on AG investment across 149 *drosophilid* species, utilizing female remating interval as a proxy for PCSS. Utilizing dry testes mass, we further examine potential trade-offs between spermatogenesis and seminal investment as it relates to PCSS. This work, coupled with ongoing comparative evolutionary proteomics, constitutes the first integrative, comparative study of AG investment and SFP composition in relation to PCSS.

1962F **Is *ftz* a pair-rule gene in *Tribolium*?** Sofia Lopez[1], Ximena Gutierrez-Ramos[2], Leslie Pick[2] [1]Entomology, University of Maryland - College Park, [2]Entomology, University of Maryland

Genes that control segmentation are expressed during early embryonic development and work together to establish the basic body plan. *fushi tarazu* (*ftz*), first identified in *Drosophila* as a pair-rule gene (PRG), is expressed in 7 stripes in the primordia of alternate segments along the anterior-posterior axis. *ftz* mutants develop as inviable truncated embryos, failing to form the regions in which *ftz* would have been expressed. In the red flour beetle, *Tribolium castaneum* (*Tc*), the *ftz* ortholog is expressed in a pair-rule manner but its function does not appear to be conserved. Previous experiments examining a large genomic deletion and RNA interference (RNAi) targeting *Tc-ftz* did not reveal pair-rule-like defects, suggesting that *ftz* is not a PRG in *Tribolium*. To definitively establish whether *Tc-ftz* functions as a PRG in this species, we employ direct parental CRISPR (DIPA-CRISPR), which has allowed efficient gene editing by injecting adult females with Cas9 and gRNAs (Shirai et. al, 2020). For this technique, CRISPR components must be injected during vitellogenesis to allow uptake into oocytes. To determine the best time of injection, we dissected ovaries from 2 to 7 day-old adult females. We found that day 4 to 7 females had more spherical and elongated oocytes that indicated initiation into the vitellogenic phase, and were chosen for injection. Two gRNAs targeting separate regions of *Tc-ftz* were injected with Cas9 protein into 5-day old females to induce directed cleavage and NHEJ. Offspring were screened by PCR with primers flanking the *Tc-ftz* targeted regions to identify heterozygotes with short indels at either of the cleavage sites. In parallel, to understand how *Tc-ftz* is regulated, we are isolating cis regulatory elements that direct pair rule expression. We utilized publicly available datasets - RNA-seq and FAIRE-seq - to identify open chromatin around *Tc-ftz* during early embryogenesis, which are likely to include regulatory sequences. To test these sequences, we generated reporter gene constructs with candidate cis-regulatory sequences upstream of the *Tc-ftz* promoter and tGFP, inserted into piggyBac vectors. After insertion into the *Tribolium* genome via embryo injection and establishment of stable lines, embryonic expression patterns will be assessed. The results of these concurrent functional and gene expression assays will reveal any contributions that *Tc-ftz* provides for segmentation and insight into its genetic regulation.

1963F **Phylogenetic comparative approaches give insight into the ecological selective pressures acting on mammalian retinas** Emily Kopania, Nathan Clark University of Pittsburgh

Vision is important for fitness in many species, but there is variation in visual acuity and the cellular structure of the retina across species. Many vertebrates have specialized high visual acuity regions in the retina defined by the density of retinal ganglion cells. Examples include the *area centralis* (high density region), horizontal streak (high density region elongated across the retina), and fovea (high density region surrounding a pit in the retina). Many have hypothesized ecological conditions that select for these specializations. For example, the *area centralis* may be used by predators for prey detection, and the horizontal streak may be associated with horizon-dominated environments (e.g., ground foraging). Despite the adaptive

potential of these specializations, these hypotheses have not been tested in a phylogenetic framework. To address this, we compiled publicly available retinal specialization phenotypes and ecological data from 83 species and overlayed these traits on a well-resolved mammal phylogeny. We found that both the horizontal streak and *area centralis* likely evolved repeatedly in mammals, and that many species have multiple specializations, such as an *area centralis* within a horizontal streak. We then used a maximum likelihood framework to test if specializations are associated with different ecological traits after controlling for phylogeny. We did not find evidence that the *area centralis* is associated with predators, casting doubt on a popular hypothesis for the role of the *area centralis*. However, we did find that the horizontal streak is significantly associated with ground foragers. Some have proposed more specific hypotheses for the function of the horizontal streak, including predator detection in grazing prey species. Therefore, we tested if the horizontal streak is associated with ground foraging herbivores less than 100kg, as these are putative prey species. We found that the horizontal streak is significantly associated with small, ground-foraging herbivores, specifically when we refined our analysis to species with only a horizontal streak. These results reveal the importance of considering evolutionary history and the complexity of retinal specialization phenotypes when investigating their potential functions. This study performs the first phylogenetic comparative test of long-standing hypotheses regarding the function of high acuity retinal specializations, thus giving insight into the ecology and evolution of visual systems.

1964F **Supergene evolution via allele-specific autoregulation** Nicholas VanKuren, Darli Massardo, Sofia Sheikh, Claire Fu, Wei Lu, Marcus Kronforst Ecology & Evolution, University of Chicago

Complex phenotypes require the coordinated action of many genes across development, yet many species can develop multiple discrete, alternate phenotypes. Recent work shows that development of such polymorphisms is often controlled by alternate supergene alleles, sets of tightly-linked mutations in one or more genes that together produce a complex phenotype. However, the process by which supergenes evolve and the mutations that cause functional differences between supergene alleles remain essentially unknown. *doublesex* is the master regulator of insect sexual differentiation but has evolved into a supergene in multiple *Papilio* swallowtail butterflies, where divergent *dsx* alleles control whether females develop distinct non-mimetic or mimetic wing color patterns. Our previous work in *Papilio alphenor* showed that the mimetic *dsx* allele gained a unique spatiotemporal expression pattern in developing wings, suggesting that the *dsx* supergene evolved allele-specific *cis*-regulatory architectures. Here we characterized the *cis*-regulatory architecture of the *dsx* supergene by identifying *cis*-regulatory elements (CREs), promoter-CRE interactions, and using CRISPR/Cas9-mediated knockouts. Hi-C and ATAC-seq showed that *dsx* is regulated solely by CREs within *dsx* introns, and that each allele has characteristic promoter-CRE loops. Seven of 31 *dsx* CREs active in developing wings (22.5%) were unique to the mimetic allele, while four (12.5%) were unique to the non-mimetic allele. In addition, Dsx CUT&RUN showed that over half of *dsx* CREs are strongly bound by Dsx itself and differentially bound in mimetic and non-mimetic female wings. At least three of these CREs are necessary for mimetic allele expression, as CRISPR/Cas9-mediated knockouts of these mimetic CREs caused females to develop non-mimetic color patterns. Our results thus provide strong evidence that the evolution of novel auto-regulatory interactions played a central role in the evolution of *Papilio* supergene mimicry polymorphisms and suggest a general mechanism for supergene evolution and function in other systems. The evolution of complex polymorphisms may therefore require simple modular changes to otherwise general developmental genes.

1965F **Biodiversity genomics of asexual and anhydrobiotic nematodes in arid and hyper-arid environments** Philipp H. Schiffer, Ann-Marie Waldvogel Institute for Zoology, University of Cologne

Deserts are among the most hostile environments to life on earth. Yet, some animals have adapted to survive and diversify under extreme water limited conditions. Evolutionary strategies for this include parthenogenesis enabling the exploration of transient ecological opportunities by offspring of a single individual, and cryptobiosis enabling organisms to wait for abiotic conditions to become favourable. Using biodiversity genomic assays in an EcoEvoDevo framework with latest sequencing methods it is now possible to study genomes of single minute invertebrates, as well as population structures in many samples from extreme environments to understand the evolutionary strategies of different species.

Here, I will present our genomic, population genetic, and ecological functional analyses of the linked evolution of these traits in nematodes from the hyper arid Atacama in comparison with the younger, more dynamic Namib desert, and the Australian Outback as a comparator system. We have established methods to conduct our genomic, and population genomic assays on-site in these remote areas and are engaged in training local students, and communities to study biodiversity on their territories.

We find locally diverse nematode species assemblages in the desert systems. In accordance with evolutionary theory these are dominated by sexual species, but heterozygosity through polyploidy appears provide an adaptive advantage to parthenogens. We observe species to employ anhydrobiosis, a mode of cryptobiosis, in different ways, to either revive fast or more slowly upon water availability.

We also recently assembled the genome of a triploid parthenogenetic *Panagrolaimus* revived from another extreme environment, the Russian permafrost. Analysing it genetically and biochemically in comparison to *C. elegans* we find resemblances in the genetic mechanisms the model organism's dauer larva and this nematode use for prolonged dormancy.

Using the CRISPR/Cas system we are now able to study parthenogenesis and cryptobiosis in *Panagrolaimus*, allowing us to understand these traits on a genetic level comparing species with different modes of reproduction and from different extreme environments.

1966F **Evolutionary Origins and Developmental Repatterning of a Morphological Novelty, the Pronotal Helmet of Treehoppers** Savanna Brown[1], Cera R Fisher[2], Elizabeth Jockusch[1] [1]Ecology & Evolutionary Biology, University of Connecticut, [2]Velsera

The evolution of morphological novelty is one major driver of species diversification. In arthropods, novel structures such as wings have repeatedly evolved as outgrowths of the body wall. One particularly enigmatic structure is the "helmet" of treehoppers, formed by a highly elaborated pronotum. One approach to investigating the origin of novelty is to characterize the development of novel structures in comparison with other body regions, as well in comparison with homologous structures of other species. Contrary to expectations of transcriptional similarity of serial homologues, our data showed that the transcriptional profile of the pronotum in the treehopper *Entylia carinata* resembles that of the wing much more than that of its serial homologue. This supports the hypothesis that the evolution of the treehopper helmet is the result of striking co-option of the wing patterning network. Furthermore, it raises questions about whether the wing patterning network extends its influence beyond wings alone, with potential involvement in the organization of bilayered epithelia throughout the body. Through RNA sequencing of several diverse treehopper species, a close, helmetless relative *Homalodisca vitripennis*, and a more distant hemipteran relative, *Oncopeltus fasciatus*, we further tested these hypotheses. We predicted that transcriptional profiles of other treehopper species will resemble that of *Entylia carinata*, showing clustering of the pronotum and wing. In tandem, we predicted that the transcriptional profiles of the helmetless but closely-related leafhopper species Homalodisca vitripennis will mirror that of distantly related *Oncopeltus faciatus*, with the clustering of the pronotum and its serial homologue. Preliminary analyses are consistent with these predictions, suggesting that the origin of this unique patterning of the pronotum aligns with the morphological origin of the helmet itself. Second, we predicted that the transcriptional profile seen in the metamorphic treehopper instar, when the pronotal tissue becomes bilayered and folds into the helmet structure, would differ from that of earlier instars. Preliminary data follows this prediction as well, aligning with the hypothesis that similarity between the patterning networks underlying helmet and wing development could be due to the shared bilayered nature of these tissues. By unraveling the genetic mechanisms governing the formation of the novel helmet of treehoppers, we gain comparative insights into the developmental patterning of bilayered epithelia and a deeper understanding of the evolutionary processes driving morphological innovation.

1967F **The DNA Damage Response gene chk2 regulates reproductive asymmetry across insects** Arjuna Rajakumar[1,2], Claire Ramsay[2], Paul Lasko[2], Ruth Lehmann[1], Ehab Abouheif[2] [1]Whitehead Institute for Biomedical Research, [2]Biology, McGill University

Eusociality is the highest level of social organization in the animal kingdom. A hallmark of eusocial groups is the evolution of a reproductive division of labor between a reproductive queen and non-reproductive worker caste that establishes, in essence, a germline-soma distinction within the colony. Remarkably, despite the evolution of reproductive and non-reproductive castes over 100 million years ago, the worker caste of the vast majority of the over 16 000 ant species retain developmentally plastic ovaries. Within a colony, workers with developed ovaries threaten social organization, and therefore, worker reproduction must be constantly regulated to maintain colony homeostasis. To this end, ant colonies have evolved multiple reproductive constraints within the germline of the worker caste that reduce their reproductive capacity, rendering them functionally sterile. However, how reproductive constraints are developmentally regulated is unknown. Here we show that chk2, a core signaling kinase in DNA Damage Response pathway, regulates multiple reproductive constraints simultaneously, and through its latent capacity to induce caste-specific phenotypes in solitary organisms, was co-opted to facilitate the evolution of reproductive division of labour in ants. Specifically, we discovered in the carpenter ant *Camponotus floridanus* that chk2 activity is increased in the germariums and oocytes of workers compared to queens and demonstrate that inhibition of chk2 in workers results in an up-regulation of numerous reproductive traits, including increased germ cell specification, reproductive activity (number of oocytes per ovariole), and reproductive fitness (percentage of viable to non-viable oocytes). Conversely, we show that overexpressing chk2 in the solitary fruit fly *Drosophila melanogaster* induces reproductive constraints (specification of fewer germ cells and mislocalization of germplasm) similar to those we observe in worker ants. Overall, our findings highlights the power of emerging and model comparative studies to tackle fundamental questions in biology.

1968S **Characterizing actin structures during tube formation in *D. melanogaster* egg chambers** Luana Paleologu[1], Celeste Berg[2] [1]Genome Sciences, University of Washington, [2]University of Washington

The folding of epithelial sheets, processes known as wrapping and budding, are the foundation for creating many tubular organs in nearly all multicellular organisms. Nevertheless, there is much to understand about the molecular mechanisms that drive cell shape changes and tissue folding. The Berg lab uses genetic and imaging techniques to understand tube formation in *D. melanogaster* egg chambers. The egg chambers form tubes that fill with chorion protein, creating eggshell structures called dorsal appendages that help embryos receive adequate oxygen. The egg chamber of *D. melanogaster* serves as a good model system to study tubulogenesis since the tissue folding and cellular shape changes during dorsal appendage formation are well understood. There are two fundamental processes that occur early in dorsal appendage formation that have been well-characterized in other systems: apical constriction and zippering. While we have a strong understanding of how cells are moving during these processes, we do not know how the cytoskeleton is coordinating shape changes, and by what signaling mechanisms these cytoskeletal dynamics are regulated. To assess these processes, we are using phalloidin-stained fixed tissue to characterize actin structures using STED microscopy, as well as live imaging of actin and myosin to characterize dynamics. These approaches revealed dynamic basal actin throughout tube formation in addition to expected apical structures. In addition, we have observed spherical, basal actin structures (50-300 nm) developing late in dorsal appendage formation. Recent studies have pointed to the importance of exosomes and other transport vesicles in aiding cell migration, leading us to hypothesize that these actin-based spherical structures may be implicated in tube elongation. Here we present a study of the changes in the actin cytoskeleton during dorsal appendage formation with a focus on how this remodeling drives cell shape change and movement.

1969S **A genomic hotspot of diversifying selection and structural change in bats (Chiroptera)** Robert S Cornman U.S. Geological Survey

Previous work found that numerous genes positively selected within the hoary bat (*Lasiurus cinereus*) lineage are physically clustered in regions of conserved synteny. Here I report an expanded analysis utilizing an updated *L. cinereus* genome assembly and additional bat species as well as other tetrapod outgroups. A chromosome-level assembly was generated by chromatin mapping and made available by DNAZoo (www.dnazoo.org). The genomic organization of orthologous genes was extracted from annotation data for multiple bat species as well as other tetrapod clades for which chromosome-level assemblies were available from the National Center for Biotechnology Information (NCBI). Tests of branch-specific positive selection were performed for *L. cinereus* using PAML. For comparison, tests were also performed with the Hyphy package and for more inclusive clades within vesper bats (Chiroptera: Vespertilionidae). Fourteen genes exhibiting significant diversifying selection in the *L. cinereus* lineage were clustered within a 15-Mb genomic window. Evaluation of additional phylogenetic branches within Vespetilionidae suggests that the majority of these genes began diversifying under positive selection prior to the divergence of the *L. cinereus* branch. Ten of the 14 genes are landmarks of two distinct blocks of ancient synteny that are not linked in other tetrapod clades. Vespertilionids are further distinguished by frequent structural rearrangements within these synteny blocks that are rarely observed in other Tetrapoda. Patterns of gene order and orientation among bat taxa are incompatible with phylogeny as presently understood, implying parallel evolution or subsequent reversals. In the bat family Phyllostomidae, a switch from pericentric to subtelomeric organization of the orthologous genes coincides with a strong increase in GC content of approximately 20 percentage points, a pattern not seen in any other tetrapod groups surveyed. This study confirms and further localizes a genomic hotspot of protein-coding divergence within Vespetilionidae, one that concomitantly exhibits an increased tempo of structural and compositional change. Most genes in the two synteny blocks have elevated expression in brain tissue in model organisms, and genetic studies implicate the selected genes in cranial and neurological development, among other functions.

1970S **Testing the function of the ebony gene in Oncopeltus fasciatus with CRISPR and RNAi** Veronika Valverde Jimenez, Leslie Pick, Katie Reding University of Maryland College Park

Oncopeltus fasciatus is a model species for the insect order Hemiptera (Chipman, 2017). CRISPR can be performed on this model organism (Reding & Pick, 2020), but visible markers are needed for genetic analyses. The *Of-vermillion* gene is the only visible marker, with homozygous mutants having bright red eyes, clearly different from wild type black eyes. CRISPR co-mutation targeting of *Of-vermilion* has proven useful to detect unlinked mutations at other loci. Since *vermilion* is X-linked in *Oncopeltus*, one quarter of all G1 progeny are not informative with regard to G0 germline mutation. Mutation of the *ebony* gene causes expansion of black melanin pigment deposition in the cuticle of many insect species. Due to this easily observable phenotype in *ebony* homozygotes, *ebony* is an ideal marker for CRISPR co-mutation: G1 progeny can be screened for the clearly visible *ebony* phenotype, and molecular screening at the locus of interest can be concentrated on lines that yield *ebony* progeny. To assess the role of *ebony* in *Oncopeltus*, nymphal and embryonic *Of-ebony* RNAi was performed. This resulted in additional black pigment present on the dorsal and ventral thorax and head. Further, gene knockdown did not appear to reduce viability at either developmental stage, suggesting we will be able to generate a stable *ebony* mutant line. To do so, we are using CRISPR/Cas9 genome editing. Four different guide RNAs were selected based on their specificity to the *Of-ebony* locus. *Oncopeltus* embryos were injected with gRNA-A and Cas9 mRNA, which resulted in a hatch rate of 42.29% (n =

584). The development of these embryos was monitored; 61 adults in the G0 population exhibited a mosaic *ebony* phenotype of varying amounts of expansion of melanin on the head and thorax. G0s were sorted by mosaic or wild type phenotype, and all mosaics were allowed to randomly mate. 139 mosaic G1 offspring were then screened for heterozygosity using a heteroduplex mobility assay, and 6 appeared to be heterozygous. Crosses to establish *Of-ebony* homozygous lines are in progress. The other three gRNAs will be evaluated for off-target effects and efficiency to select the best gRNA for use in CRISPR co-mutation schemes. These preliminary results suggest we will be able to generate a stable *ebony* mutant line, expanding the genetic markers for *Oncopeltus*.

1971S **Developmental transcriptomics in *Pristionchus* reveals the timing, responsiveness, and evolution of a plastic trait** Shelley J Reich, Samantha Nestel, Michael S Werner School of Biological Sciences, University of Utah

The nematode *Pristionchus pacificus* exhibits phenotypic plasticity in mouth form and has emerged as a model system for studying developmental plasticity. These worms can develop either a narrow, bacterivore mouth with a single tooth or a wide, omnivore mouth with two movable teeth that enables predation on other nematodes in addition to feeding on microbiota in the environment. Mouth-form development is sensitive to environmental conditions and biased by the worm's genetic background. Prior studies have uncovered several genes which are required for alternative mouth-form phenotypes. We collated these data into a gene regulatory network (GRN) and performed developmental transcriptomics across different environmental conditions, genetic backgrounds, and mouth-form mutants to identify 1) which genes in the network are environmentally responsive, 2) when during development are they responsive, and 3) how these patterns change over evolutionary time. We found that expression of only a subset of previously identified mouth-form genes is sensitive to environmental conditions. The timing of their expression relative to a critical developmental window informs their role in executing the mouth-form decision. A comparison between strains and species with different mouth-form biases reveals a pruning of the GRN across evolution. Surprisingly, our analyses suggest different roles for the two previously identified switch genes, *eud-1* and *seud-1/Ppa-sult-1*. The former appears to be an environmental sensor, while the latter sets the default mouth form for a given genetic background. A comprehensive analysis of all sample types (environmental, mutant, strain, and species) identifies common pathways involved in regulating mouth-form development. Collectively, our temporal analyses provide a framework for the genetic regulation of developmental plasticity.

1972S **MSS-related family of glycoproteins in *Caenorhabditis* contribute to male sperm competitive fitness via unknown mechanisms** Asan Turdiev[1,2], Jillian Manning[1], Eric Haag[1,2] [1]Biology, University of Maryland, [2]Biological Sciences Graduate Program, University of Maryland

Sperm competition is a major component of sexual selection and is present in all major animal groups. Research from the past decades on several animal models, including flies, mice, and nematodes, has led to the identification of genes with apparent roles in sperm competitiveness. Their molecular mechanisms are generally unknown, but they appear distinct from core fertilization factors. The genetically dispensable nature of sperm-competition genes for reproduction makes them more amenable to genetic manipulation than fertilization genes, yet they may reveal general lessons about gamete biology. Using comparative genomics, the Haag lab previously identified genes encoding small sperm glycoproteins present in outcrossing species but consistently lost in hermaphrodites. These comprise the Male Secreted Short (MSS) family. Restoring *mss* to males of the self-fertile *C. briggsae* via transgene from a close male-female relative *C. nigoni* led to a large increase in male mating success in competitive contexts. While *mss* has been lost in selfing species, *mss-related proteins* (*msrps*) show evolutionary conservation across all *Caenorhabditis*. Here we asked whether *msrps* are retained because they are required for baseline fertility. Our research on *C. briggsae* shows that MSRPs are indeed expressed in sperm. Deletion of a cluster of *msrp* does not affect the mutant hermaphrodite's brood size nor the mutant male's natural ability to compete with WT hermaphrodite's sperm post-mating. However, under competitive settings where male sperm compete for fertilization, MSRP$^-$ sperm display a fitness defect. The precise biological roles of MSRPs on the male sperm inside the female's reproductive tract is the focus of ongoing research.

1973S **Characterizing the composition and morphology of the germ plasm in the wasp *Nasonia vitripennis*** Allie Kemph[1], Jeremy Lynch[2], Alexey Arkov[3], Kabita Kharel[3], Samuel Tindell[3] [1]Biological Sciences, University of Illinois at Chicago, [2]University of Illinois at Chicago, [3]Murray State University

Specification of germ cell fate during embryogenesis is an essential process in sexually reproducing organisms to ensure the correct transmission of parental genetic information to offspring. In many cases it involves germ plasm, a specialized cytoplasmic organelle composed largely of mRNA and RNA-binding proteins. We are working to characterize a uniquely large and dynamic germ plasm found in the wasp *Nasonia vitripennis*, called the oosome (~25um). During early embryogenesis the oosome is initially attached at the posterior cortex, but quickly detaches and migrates a significant distance anteriorly, returns to the posterior cortex where it induces single large pole budding event involving multiple nuclei. This contrasts with germ

plasm in *Drosophila,* which consists of many small (0.2-0.5um) “polar granules”, which are static at the posterior cortex until individual nuclei induce pole cell budding. In order to understand the molecular and structural differences between polar granules and the oosome, we have undertaken a detailed analysis of the oosomes composition and ultrastructure using super-resolution microscopy and TEM. Oosome ultrastructure is characterized by a complex mesh-like network of electron dense material that forms numerous pockets and channels. This network also incorporates and interacts with numerous unusual membrane-bound organelles, and excludes mitochondria. Fluorescent super-resolution microscopy has revealed some of the molecular basis for the unique features of the oosome. We found that highly enriched oosome mRNAs are arranged in a complex pattern consisting of highly concentrated puncta connected by a meshwork of lower concentration regions. Less enriched mRNAs are found in more dispersed puncta along the networks of high-level transcripts. Conserved germ plasm proteins also show unexpected patterns. Tudor accumulates in a shell-like domain encapsulating the oosome, while Oskar and Vasa are found in puncta that do not appear to nucleate mRNA localization. Understanding how these unusual arrangements arise and are maintained will provide unique insights into how mRNA and protein can be deployed to form functional membraneless organelles.

1974V **The Evolution of fem-1 activity in Caenorhabditis** James F Kennedy[1], Ronald Ellis[2] [1]Molecular Biology, Rowan GSBS, [2]Rowan GSBS

Hermaphrodite sex determination is shared between the Caenorhabditis species C. elegans, C. briggsae, and C. tropicalis, but this trait is thought to have occurred independently. The core pathway that makes this determination shares large similarity in the Caenorhabditis genus among which are the FEM complex proteins: FEM-1, FEM-2, and FEM-3. These proteins were discovered in C. elegans where corresponding genes are necessary for male sex determination as evidenced by null mutants producing feminizing mutations in the soma and germline in both XX and XO animals. This phenotype is not shared in C. briggsae where fem-2 and fem-3 null mutants in both XX and XO animals are hermaphrodites. This difference suggests a divergence in the role of the FEM complex in these two species. My research began with developing a fem-1 null mutant by CRISPR/Cas9 injection in C. briggsae.

The mutant strain of fem-1(v508) showed an identical phenotype to C. briggsae fem-2 and fem-3 null mutants producing fertile XX and XO hermaphrodites. XO hermaphrodites were verified through RT-PCR of her-1 mRNA and unc mutant genetic crosses. To place fem-1 in the sex determination pathway, double mutants of this gene with its direct upstream (tra-2) and downstream (tra-1) genes were produced. The expected results were received with tra-1(v181); fem-1(v508) mutants being XX males and tra-2(nm1); fem-1(v508) mutants being XO hermaphrodites. While the FEM proteins were expected to act as a complex, a null triple mutant of fem-2(nm27); fem-3(nm63) fem-1(v517) was produced by CRISPR/Cas9 of fem-1 in an existing fem-2; fem-3 double mutant. This mutant has an identical phenotype to individual null mutants.

A complex relationship between the FEM proteins and germline development is suggested by oogenesis in tra-1; fem double mutants in C. elegans. This was replicated in C. briggsae by germline scoring of tra-1(v181) mutants with corresponding fem genes. A spermatogenesis to oogenesis switch was seen in these tra-1 males. This result suggests that the fem genes serve an unknown downstream function in both C. elegans and C. briggsae.

The epistatic interactions of C. tropicalis between fem-1 and its upstream (tra-2) and downstream (tra-1) genes in the sex determination pathway will be determined with created null mutants by CRISPR/Cas9. These null mutants (tra-1 and tra-2) are XX males. It is expected that the somatic and germline phenotypes of double mutants will show similarity to C. elegans.

1975V **Identification of changes in regulatory sequence underlying the gain of polka-dotted pigmentation pattern in *Drosophila guttifera*** Takumi Karasawa[1], Shigeyuki Koshikawa[2] [1]Division of Biosphere Science, Hokkaido University, [2]Division of Biosphere Science, Graduate School of Environmental Science, Hokkaido University / Faculty of Environmental Earth Science, Hokkaido University

Evolution has produced a variety of traits throughout the history of life. A central goal in evolutionary developmental biology is to reveal how diverse traits are newly gained. Recent studies have shown that the co-option of pre-existing genes or gene regulatory networks is important in the gain of new traits. One factor that can cause co-option is changes in *cis*-regulatory elements, but what kind of changes in sequence occur in the co-option are still poorly understood.

Drosophila guttifera has a polka-dotted pigmentation pattern, which is unique to this species on its wing. This pigmentation pattern is known to have been acquired via the co-option of a developmental gene, *wingless*. The pigmentations are formed around longitudinal vein tips, intersecting points of longitudinal veins and crossveins, and campaniform sensilla, and *wingless* is expressed in the center of these positions during the pupal stage. In *Drosophila melanogaster*, which does not have a pigmentation pattern on its wing, *wingless* is expressed only in the crossveins in the pupal wing. A previous study has identified a *cis*-regulatory region that regulates expression in the crossveins and longitudinal vein tips (gutCVT-core) and

showed that this *cis*-regulatory activity is gained via changes in the ancestral regulatory element, which activates expression only in the crossveins. However, the changes in sequence that brought expression in the longitudinal vein tips are still unknown.

In this research, we compared the sequence and the regulatory function of gutCVT-core with the homologous region of a close relative, *Drosophila angularis* (angCV-core), and identified the changes in sequence that brought the *wingless* expression in the longitudinal vein tips.

We compared the sequence of gutCVT-core and angCV-core and identified several blocks of sequence that mismatch between the regulatory regions. We then made chimeric regulatory sequences in which each mismatched sequence in gutCVT-core is replaced with the corresponding sequence in angCV-core. Testing the regulatory function of these chimeric sequences by EGFP reporter assay, we identified several sequences that function in driving expression in the longitudinal vein tips and are newly gained in gutCVT-core. Our results suggest that not a single, but multiple changes in sequence were required to cause the co-option of *wingless*.

1976V **Identifying co-factors for TRA-1 activator function** Jibran Imtiaz, Youngquan Shen, Ronald Ellis Molecular Biology, Rowan-Virtua STBES

Gli proteins are conserved transcription factors with five zinc fingers. The first one discovered was Cubitus interruptus (Ci) in *Drosophila*. Humans have three Gli proteins, which play a key role in many tissues and organ development, and their misregulation causes some cancers and birth defects. Human Gli proteins and Ci are all involved in Hedgehog (Hh) signaling pathways. However, what other co-factors they utilize, which co-factors are essential for activator function, and how they interact with them remain poorly understood. We are analyzing co-factors of TRA-1, the only Gli protein in *Caenorhabditis*, which is even conserved in the distantly-related nematode *Pristionchus pacificus*. Like all Gli proteins, the TRA-1 zinc fingers are highly conserved and TRA-1 is cleaved to form a transcriptional repressor. In nematodes, TRA-1 controls all sexual fates, largely by repressing genes needed for male development, and it also plays a central role in self-fertility. These traits make it easy to assay mutant phenotypes. Furthermore, worms lack classical Hedgehog signaling, so study of nematode TRA-1 should reveal other types of regulation.

Our lab has shown that full-length TRA-1 can work as an activator and promote spermatogenesis, and that the mutation *cbr-tra-1(v48)* disrupts this process and prevents spermatogenesis. We suspect that regulation of TRA-1 activator plays a major role in the evolution of hermaphrodite spermatogenesis in nematodes. Because *v48* was isolated in a classical EMS mutagenesis, we recently made other activator mutations to confirm that all of its phenotypes were due solely to the alteration of TRA-1.

TRA-1 activator is likely to interact with a diverse set of co-factors, whose activities might help determine whether specific targets are activated or repressed. Our results have shown that TRR-1 works directly with TRA-1 activator to promote expression of the *fog-1* and *fog-3* genes. We also purified TRA-1::N-OLLAS using dynabeads and are now characterizing the products obtained from these tagged worms and are preparing to analyze them with mass spectrometry. While doing so, we will look for important modifications to TRA-1 itself, as well as the precise site of cleavage that forms the repressor. In addition, we hope to identify TRA-1 co-factors and learn how they regulate Gli activity. Finally, we will see if any of these co-factors has a novel role in species that produce self-fertile hermaphrodites.

1977V **Evolution and Development of Egg Tooth Across Aminotes** Jingjing Wang Life Science Institute, Zhejiang University

Egg tooth is essential for the egg-laying amniotes to break out of the eggshells toward the end of hatching, and sheds shortly. Little is known about the mechanisms of egg tooth initiation, propagation and detachment progress from the beak/mouth part, as well as the factors contributing to the morphological diversity across species, despite their vital role. It severs a paradigm to study the evolution and developmental mechanisms that can be applied to many other important yet puzzlingly diversified body appendages. To address these questions, I collected developing embryonic egg tooth samples from a variety of oviparous amniotes that possess epithelial egg teeth composed primarily of keratins. These species include chicken, duck, emu, ostrich, crocodile, and turtle. I found that the development of egg teeth is initiated with gradual keratinization starting from a transparent bump on the beak/mouth that subsequently spreads, and merges with the complete keratinization of the beak. We test the hypothesis that the initiation of egg tooth is associated with that of subsequent keratinization of beaks in chicken. To do that, we removed the primordium of egg tooth in chicken at different stages. We find that the beak keratinization process is not affected by removal of early egg tooth primordium, but by that of the later stages. Keratinization of all beaks occurs during the late stages of chicken egg tooth development. Interestingly, our study revealed that crucial transcription factors, including *HOXC13*, *FOXN1*, and *KLF5*, play central roles in initiating egg teeth development across different species. Furthermore, the distinct variations in the unique number of alpha and beta keratin genes, as well as their

expression patterns across different species, provide pivotal insights into understanding the complex mechanisms of egg teeth development and evolution.

1981T **NCBI Datasets: an innovative resource for finding and downloading NCBI sequence and metadata for organisms across the tree of life.** Nuala O›Leary, Eric Cox, Terence Murphy, Valerie Schneider NCBI, National Institutes of Health

The National Center for Biotechnology Information (NCBI) maintains the largest global depository of genome sequences, annotations, and associated metadata, encompassing a wide variety of life forms. However, the burgeoning size and complexity of genomic information pose significant obstacles for researchers attempting to locate and obtain complete genome datasets in user-friendly formats suitable for their specific analytical processes. Moreover, there is a pressing need for data infrastructures and sharing protocols that comply with the FAIR principles, ensuring data is Findable, Accessible, Interoperable, and Reusable. Toward this goal, NCBI introduces NCBI Datasets, a new resource that develops web, command-line, and API interfaces for accessing NCBI sequence data that are intuitive and user-friendly. Datasets delivers data as a coherent data package including sequence, annotation, and metadata for assembled genomes, genes, and orthologs. This presentation will highlight the most recent advancements in Datasets and illustrate and highlight how NCBI Datasets is supporting CGR is an NIH-funded, multi-year NLM project to establish an ecosystem to facilitate reliable comparative genomics analyses for all eukaryotic organisms in collaboration with the genomics community.