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ABSTRACT BOOK

GENETICS GSA G3 Genes | Genomes | Genetics

1 **Function of satellite DNA in Drosophila spermatogenesis** Yukiko M Yamashita Whitehead Institute for Biomedical Research, MIT, HHMI

Satellite DNA is tandem-repetitive DNA that constitutes a large fraction of the genome. Due to their non-coding nature and lack of sequence conservation between species, the satellite DNA has been generally regarded as junk. However, we have recently shown that satellite DNA plays key roles in various processes, including the maintenance of nuclear architecture and expression of Y-linked fertility genes. Loss of satellite DNA binding proteins in mitotic cells leads to defective bundling/encapsulation of chromosomes into a single nucleus, causing micronuclei. Regulation of satellite DNA expression is critical in spermatocytes, where Y-linked fertility genes that have megabase-sized satellite DNA-containing introns must be expressed in a spatiotemporally coordinated manner. The latest results on how satellite DNA biology contributes to the processes of spermatogenesis will be discussed.

2 **Adipocyte-Derived Amino Acid Storage Proteins Regulate Distinct Steps of Oogenesis** Robert C Eisman, Lesley Weaver Indiana University

Nutrient availability, stress, age, and other physiological changes influence stem cell lineages. Adult stem cell populations mediate tissue homeostasis, which is intimately linked to the physiology of an organism. Multiple endocrine organs (such as adipose tissue, muscle, and the gut) relay physiological status to other organs (such as the ovary) through circulating factors to influence their function. However, disruption of signaling from endocrine organs often leads to pathologies such as obesity, increased cancer risk, or organ failure. My lab is interested in understanding how organs communicate their physiological status to one another to maintain optimal tissue function, including oogenesis.

I previously found that the nuclear receptor, Seven Up (Svp) is required in adult female adipocytes to regulate distinct steps of oogenesis. RNA sequencing analysis from purified fat bodies and oenocytes showed that genes related to innate immunity and amino acid storage are significantly decreased in the fat body when *svp* is reduced. Amino acid storage proteins are hexamerins that are primarily synthesized in the fat body during the larval feeding period and reabsorbed by the fat during metamorphosis to provide building blocks for adult tissues. My recently established lab has found that adipocyte-derived amino acid storage proteins are required in adult females to regulate distinct steps of oogenesis. We found that adipocyte-specific loss of the amino acid storage protein receptors *Fat Body Protein 1* (*Fbp1*) and *Fbp2* significantly decreases GSC number. Furthermore, the amino acid storage proteins Lsp1a, Lsp1b, and Lsp1g are required in adipocytes for survival of vitellogenic follicles. We are currently testing whether adipocyte-derived amino acid storage proteins are either: 1) secreted into the adult hemolymph and transported to the ovary to regulate distinct stages of the GSC lineage; or 2) required cell autonomously in the adult fat body to remotely control ovarian function by catabolizing amino acid storage proteins. Our work will provide insight to how adipose-derived secreted factors influence oogenesis and ovarian tissue homeostasis.

3 **Molecular kinetics of gene regulation and nuclear organization during development** Mustafa MirCell and Developmental Biology, University of Pennsylvania and Children's Hospital of Philadelphia

During early development, gene expression patterns progressively emerge as cell fates are determined. The regulation of embryonic patterning at the transcriptional level occurs across a broad range of spatial and temporal scales. These scales range from the molecular scale dynamics of regulatory proteins binding to genomic loci to activate and repress genes, to the organization of chromatin and distribution of proteins within nuclei at sub-micron to micron scales, to the emergence of domains of gene expression at the scale of hundreds of microns. I will discuss the application of high-resolution light-sheet microscopy, single molecule tracking, live imaging of transcription, and new analysis approaches, to functionally bridge data acquired across this wide range of spatial and temporal scales. I will show how combining imaging data with biophysical analyses and systematic mutagenesis of key transcription factors allows us to dissect how protein-protein interactions influences the ability of transcription factors to efficiently find and bind their genomic targets. I will present data on how transient assemblies of transcriptional regulators called hubs, mediate transcription factor binding by regulating the frequency of interactions rather than their stability. I will discuss how applying the new quantitative approaches we developed has changed our understanding of how the nucleoplasm is functionally organized in space and time to achieve the robust patterns of transcription necessary for proper development of Drosophila embryos.

4 **Why flies like getting buzzed: Neuromolecular mechanisms for motivation** John Hernandez[1], Tariq Brown[1], Emily Petruccelli[2], Kristin M Scaplen[3], Amanda Waterman[1], Reza Azanchi[1], Karla R Kaun[41]Brown University, [2]Southern Illinois University Edwardsville, [3]Bryant University, [4]Neuroscience, Brown University

Investigating how drugs of abuse affect molecular mechanisms within reward memory circuits is key to understanding how cravings are acquired and expressed. Combining forward genetics, transcriptomic and detailed circuit mapping approaches in *Drosophila*, we've shown how alcohol influences the highly conserved Notch and JAK/STAT signaling pathways to alter gene expression required for memory formation. This coincides with dynamic alternative splicing within cue-encoding neurons. These molecular changes influence a dynamic shift from circuits that form memories for both the aversive and rewarding properties of alcohol, to circuits that initiate cue-induced behavioral responses and escalation of alcohol self-administration.

5 **Genetic variation in *P*-element dysgenic sterility: how flies put up with invading genomic parasites** Erin S Kelleher Biology and Biochemistry, University of Houston

Transposable elements (TEs) are vertically transmitted genomic parasites that comprise up to 80% of eukaryotic genomes. As TEs self replicate in germline cells, ensuring their persistence and spread through host populations, they introduce double stranded breaks that compromise genome integrity. This mutagenic threat is particularly pronounced when a host is invaded by a new TE, for which it lacks regulatory small RNAs.The horizontal transfer of TEs between distantly related species happens recurrently over evolutionary timescales, representing a ubiquitous challenge faced by all organisms.

My research group harnesses the historic invasion of *P*-elements into *Drosophila melanogaster* to reveal how host species respond to and survive TE invasions. These famed DNA transposons, which empowered the earliest days of genetic modification in *Drosophila*, invaded natural populations in the 1950s and rapidly spread worldwide. Their self-replication in germline cells causes a sterility syndrome known as hybrid dysgenesis, which in its most acute form is characterized by a total loss of germline cells. Using historic collections of *D. melanogaster* isolated before *P*-element invasion, my lab has uncovered ancestral genetic variation in hybrid dysgenesis, which arises both from differences in the facilitation of *P*-element transposition by host germline cells, and also the response of germline cells to double stranded breaks. These ancestral variants have dramatic consequences for fertility in the presence of *P*-element transposition, and likely helped ensure reproduction of wild *D. melanogaster* following *P*-element invasion.

6 **Immune Regulation of Intestinal Stem Cell Dynamics Is Essential for the Repair of Damaged Epithelia** Edan Foley University of Alberta

Intestinal stem cells generate and maintain a gut epithelial barrier that protects from toxic lumenal material. In flies, the Immune Deficiency/NF-kB pathway is a critical regulator of gut homeostasis. However, it is less clear if NF-kB directly impacts stem cell responses to damage. We consider this an important question, as stem cell dynamics dictate the efficacy of epithelial renewal, and failure to engage repair pathways significantly diminishes health and longevity. We used adult flies to examine the effects of stem-cell intrinsic NF-kB loss on tissue repair after chemical or bacterial injury. We discovered that loss of NF-kB significantly increased stem cell death, impaired the regeneration of mature epithelial cells, and greatly enhanced fly mortality, indicating an autonomous role for NF-kB in stem cell viability during acute epithelial damage. Mechanistically, we showed that the Ras-ERK pathway acts downstream of NF-kB to maintain stem cell viability and barrier regeneration. In combination, our data uncover an essential role for NF-kB/RAS-ERK signaling in stem cell viability during epithelial repair and emphasize the importance of immune signals for the ability of stem cells to navigate extrinsic challenges.

7 **Discover and Develop Your Community** Raquell Holmes[1], Tânia Reis[21]improvscience, [2]University of Colorado, Anschutz Medical Campus

In any setting, everyone plays a part in creating a welcoming, creative and caring environment. By showing up and trying something new—together as scientists—we will discover in-the-moment ways to foster belonging and develop new relationships that last well beyond Dros23.

Have you wanted to meet other scientists in our fly community? Have you wanted to foster an equitable and inclusive community?

In this plenary session, you'll be introduced to and experiment/play with collaborative approaches in fostering a culture of belonging. Together, we will explore how the cultural acts of curiosity, giving attention, insight and opportunity can develop the diversity of our scientific communities. In addition to the interactive exploration led by Dr. Raquell Holmes, you'll participate in a hosted discussion with Dr. Tânia Reis.

Dr. Reis will share her experience with changing the culture of graduate admissions at her home institution. She will provide personal examples of how a mix of knowledge and feelings (aka “wise mind”) can guide critical discussions, decrease perceived biases and support collective decision making. Dr. Reis also shares how our common scientific language can help give direction to this personal/cultural and subjective process. We will hear her thoughts about what worked and what could have worked better as her admissions committee collectively pursued replacing exclusionary approaches with strategies for equitable and inclusive admissions.

Come discover and develop our fly community as we create new ways to pay it forward.

8 **High-resolution Hi-C shows chromatin state is responsible for pairing changes in Drosophila hybrids** James G Baldwin-Brown, Nitin Phadnis, James G Baldwin-BrownBiology, University of Utah

The pairing of homologous chromosomes during meiosis is essential across all eukaryotes. Despite the discovery of pairing more than a century ago, we understanding very little about its molecular mechanisms and associated phenomena. Like most organisms, Drosophila pair their homologous chromosomes during meiosis. However, unlike most organisms, Drosophila also pair their chromosomes in somatic cells. This property has been used to study the mechanisms of pairing in the context of polytene chromosomes. Strangely, polytenes in many interspecies hybrids of Drosophila have highly reproducible regions that fail to pair interspersed among paired regions. This property of genomic regions of synapsis (proper pairing) and asynapsis (failure to pair) within the same nuclei promises to delineate genomic factors that contribute to pairing.

We used Hi-C to build a high-resolution chromosome pairing map in hybrids between Drosophila melanogaster to its sister species Drosophila simulans. Our studies provide several surprising results. Oligopaint FISH and Hi-C show that diploid nuclei pair properly even in hybrids, and that asynapsis is found only in polytene cells. These results suggest pairing mechanisms may vary in different cell types, and have consequences for inferences from pairing studies in cell culture, embryos, and polytenes. We generated a Hi-C pairing map in polytenes from hybrids’ salivary glands. This genome-wide, high-resolution, quantitative view of pairing in hybrids shows that intuitive and long-standing ideas about the determinants of pairing, such as sequence divergence, insulator sequences, etc. do not explain the patterns of asynapsis in hybrids. Instead, we found the type of chromatin was a key determinant of pairing in hybrids. In pure species, BLACK and GREEN chromatin induce somewhat low pairing. In hybrids, however, we found a very different pattern: BLACK chromatin drove high pairing, while GREEN chromatin drove low pairing. We also generated matched data from these hybrids to further dissect the roles of DNA replication and transcription, along with the role of hybrid incompatibilities in chromosome pairing in inter-species hybrids. Briefly, replication is a strong driver of pairing in pure species and hybrids, and known hybrid incompatibilities have minimal effects on pairing. Our discoveries open new avenues to understanding both the mechanistic basis of pairing and its relationship to reproductive incompatibility between species.

9 **Rapid centromere turnover in *Drosophila* gives rise to telocentric chromosomes** Cecile Courret[1], Ching-Ho Chang[2], Lucas Hemmer[1], Xiaolu Wei[1], Bryce Santinello[3], Barbara Mellone[3], Amanda Larracuente[11]Biology, University of Rochester, [2]Fred Hutchinson Cancer Center, [3]University of Connecticut

Centromeres are chromosomal structures required for faithful genome inheritance during cell division. Centromeres are typically defined epigenetically by the presence of the centromere-specific histone H3 variant, CENP-A. While centromeres typically form in repeat-rich regions of the genome, the roles of DNA sequences in centromere function are unclear. We recently revealed that all centromeres in *D. melanogaster* correspond to islands of complex DNA enriched in retroelements and flanked by tandem repeats. Each centromere is unique—the only sequence they have in common is the *G2/Jockey-3* retroelement. It is unclear if any of these sequences are important for centromere function. Here we study the evolution of centromere composition to gain insights into the role of DNA sequence in centromere biology. By combining heterochromatin-enriched genome assemblies with CUT&Tag for the CENP-A antibody and fluorescence in situ hybridization, we characterized centromere organization in three sister species: *D. simulans, D. sechellia,* and *D. mauritiana*. We discovered that *simulans* clade centromeres have similar organization as *D. melanogaster*’s: islands of complex repeat flanked by tandem repeats. However, none of the *D. melanogaster* centromere islands are conserved in the *simulans* clade. Instead, in the *simulans* clade centromeres are mainly composed of two complex satellites: *500bp* and *365bp*. Those two complex satellites are specific to the *simulans* clade, suggesting that they invaded and replaced centromeres after the split with *D. melanogaster*. In addition, we observed a second replacement event specific to *D. sechellia*. On the dot and X chromosome centromeres of *D. sechelli*a CENP-A appears to be enriched on the telomeric

sequences, revealing for the first time true telocentric chromosomes. Finally, *G2/Jockey-3* is enriched in *D. simulans* centromeres, but much less so in *D. sechellia* and *D. mauritiana.* Identifying the functional centromeric DNA gives insights into their roles in chromosome function and evolution and these results highlight the very rapid turnover of centromeric sequences among the *melanogaster* clade and are consistent with recurrent genetic conflict.

10 **Coevolution between two essential telomere binding proteins preserves chromosome end-protection**
Sung-Ya Lin[1], Hannah Futeran[1], Mia Levine[1,21]Department of Biology, University of Pennsylvania, [2]Epigenetics Institute, University of Pennsylvania

Chromosome ends pose two major threats to genome integrity: the erosion of unique DNA sequence and inappropriate DNA repair into lethal, end-to-end chromosome fusions. Specialized telomere binding proteins combat these threats by adding repetitive DNA to chromosome ends and by preventing chromosome ends from fusing. Although these telomere functions are essential and conserved in all eukaryotes, many telomere proteins are unconserved. Nearly half of *Drosophila* proteins required for telomere functions evolve rapidly under positive selection, including two essential components of the end-protection complex, HipHop and HOAP. These two proteins physically interact and recruit each other to the telomere, raising the possibility of coevolution between HipHop and HOAP. Our lab previously showed that HOAP evolves adaptively to restrict the proliferation of the telomeric retrotransposons that elongate telomeres. We hypothesize that HOAP adaptive evolution disrupts its interaction with HipHop. HipHop then coevolves to maintain its interaction with HOAP, ensuring chromosome end-protection. To test the hypothesis, we transgenically replaced the native *D. melanogaster hiphop* with its own version as a control ("*hiphop[mel]*") or a highly diverged version from its close relative, *D. yakuba* ("*hiphop[yak]*"). We discovered that *hiphop[yak]* flies are homozygous lethal at the pupal-to-adult transition and observed high rates of telomere fusions, two phenotypes reminiscent of the *hiphop* null mutant. Unexpectedly, HipHop[yak] successfully localizes to telomeres, while the *D. melanogaster* HOAP ("HOAP[mel]") is absent, suggesting that there is disrupted HipHop-HOAP coevolution. To test this possibility, we swapped *HOAP* from *D. yakuba* into the *hiphop[yak]* flies ("*HOAP[yak],hiphop[yak]*"). Remarkably, unlike HOAP[mel], HOAP[yak] localizes robustly to the HipHop[yak]-bound telomere ends and rescues both telomere end-protection from fusions and adult viability. These results support our hypothesis that HipHop coevolves with HOAP to maintain telomere integrity. We are currently mapping the HipHop[mel]-specific domains required for this vital interaction with HOAP[mel] using HipHop[mel]-HipHop[yak] chimeras. In parallel to these studies in the soma, we found that *hiphop[yak]* reduces female fertility in heterozygous flies. Like in the soma, *HOAP[yak]* restores female fertility of these heterozygotes. These results suggest that HipHop-HOAP coevolution also preserves female fertility and more generally, that HipHop evolves adaptively to "keep up with" HOAP as it evolves adaptively to restrict selfish telomeric retrotransposons. This study implicates a selfish element-induced "evolutionary cascade" that sculpts fundamental cellular processes vital for viability and fertility.

11 **Single and repetitive oligopaints probes label specifically neo-Y chromosome of *Drosophila miranda*** Henry Bonilla[1], Isabela P de Almeida[1], Mara LS Pinheiros[1], Maria D Vibranovski[1,21]Genetics and Evolutionary Biology, University of São Paulo, [2]New college for Interdisciplinary Arts and Sciences, Arizona State University

Drosophila miranda is a model species to study sex chromosome evolution due to its so-called neo-chromosomes. A fusion between an autosome and the Y chromosome produced the metacentric Neo-Y about 1.5 MYA ago whereas its homologous pair became the neo-X. Fluorescent in-situ hybridization (FISH) is a valuable resource for investigating chromosome evolution, chromosome behavior and, ultimately, for assessing the quality of genome assembly. Oligopaints represent a new generation of probes (1) that are custom-synthesized oligonucleotides, versatile and specific for targeting single-copy chromosome regions. OligoMiner (2) is a pipeline to design oligopaints, however, it was originally thought to develop single oligos. Due to the nature of the neo-Y chromosome, degenerated and similar to Neo-X chromosome, the amount of single oligos designed by OligoMiner might not be enough to paint this chromosome. To overcome this problem, we modified the conventional flow of Oligominer to obtain repetitive specific oligos, adding steps such as non-overlapping oligo selection as well as the incorporation of K-mers, secondary structure and female short-read filters to guarantee specificity to Neo-Y chromosome. We took advantage of the latest *D. miranda* genome sequence (3) and YGS (4) to identify 33,427 (0.75 hits/kb), 28,003 (0.84 hits/kb), 1,832 (0.21 hits/kb) single and repetitive oligos for the Y1, Y2 and Y3 scaffolds, respectively. Multiple FISH experiments on mitotic chromosomes revealed that our oligo libraries covered the entire Y chromosome with no off-target signal. Despite the poor density compared to previous experiments with *D. melanogaster* (1 hit/kb) (5), fluorescence was detected for single and repetitive libraries on three different locations corresponding to the given scaffolds. Interestingly, our labeling experiments showed that the two Neo-Y scaffolds, Y1 and Y2, corresponding to the Muller C element are not physically concatenated in situ. Yet, they are interspersed with the Muller D element corresponding scaffold (Y3).Our findings could explain why Muller C was

assembled in two distinct scaffolds, implying a chromosomal translocation with the pseudoobscura group Y chromosome (Muller D) during or after the Muller C fusion.

1-Beliveau BJ et al 2012 109: 21301-6 2-Beliveau B et al 2018 115:E2183-E2192 3-Carvalho B and Clark A 2013 23:1894–1907 4-Mahajan S et al 2018 16:e2006348 5-Rosin LF et al 2018 14: e1007393 12 **Rapid evolution of piRNA clusters in the *D. melanogaster* ovary** Satyam P Srivastav, Andrew G Clark, Cedric FeschotteMolecular Biology and Genetics, Cornell University

Animal genomes are parasitized by a horde of transposable elements. In their defense, animals use the piRNA pathway, which represses TEs in the germline by a specialized family of Argonaute proteins and small RNAs produced from discrete genomic regions called piRNA clusters (piCs). While positive selection is documented in genes encoding piRNA pathway components, indicative of rapid evolution driven by genetic conflict, little is known regarding piC evolution. To investigate piC evolution, we use a population genomics approach to quantify divergence of cluster activity and sequence composition across 8 inbred strains of *Drosophila melanogaster* of distinct geographical origin with high quality genome assemblies. We performed annotations of piCs and TEs and tested predictions of two previously proposed models of piC evolution – '*de novo'* and 'trap'. The *'de novo'* model posits that TE silencing is driven by small piCs derived from individual TE insertions, and the 'trap' model argues for large stable clusters composed of multiple TE insertions which can acquire defense against new TEs when they insert within the piC. It remains unclear which model best describes the evolution of piCs. Our analysis uncovers extensive variation in piC activity across strains and signatures of rapid birth and death of piCs. Using a comprehensive annotation of genome-wide TE insertions and piC landscape, we find that surprisingly few select LTR retrotransposons have given rise to majority of strain-specific piCs, suggesting specific mechanism of frequent *de novo* emergence of piCs. However, we also find enrichment of recent insertions from many active TE families in large trap-like piCs. Thus, our findings support aspects of both *'de novo'* and 'trap' models of piC evolution. We propose that these two models represent two extreme phases along an evolutionary continuum, which starts from the emergence of piCs from LTR retrotransposon insertions of select families. Such piCs occasionally expand into larger 'trap' clusters by accretion of additional TE insertions during evolution. Our results provide insights into recent, rapid evolution of active piCs in the *D. melanogaster* ovary.

13 **Cracking open an evolutionary mystery: Using the eggshell ECM to understand how cell polarity, cell adhesion, actomyosin contractility, and patterned secretion contribute to diverse ECM morphologies** Seth Donoughe, Avinash Sholevar, Audrey Williams, Ed Munro, Sally Horne-BadovinacMolecular Genetics and Cell Biology, University of Chicago

Does the evolution of morphogenesis follow predictable patterns? We discovered that in at least one highly conserved epithelium, the answer is yes. We studied the insect follicular epithelium, the somatic tissue in the ovary that surrounds each developing egg and apically secretes an extracellular matrix (ECM): the eggshell. We measured the ECM traits of this tissue in hundreds of insect species, and then analyzed these traits in an evolutionary framework to reconstruct patterns of evolution over hundreds of millions of years. This revealed that aspects of cell shape, cell packing, and cell secretion have convergently evolved many times in insects. This analysis also enabled us to formulate mechanistic hypotheses about epithelial organization and ECM patterning, and then test them with functional experiments in a model species—*Drosophila melanogaster*. We conducted a targeted RNAi and overexpression screen to identify cellular processes that can control variation in epithelial arrangement and ECM structure. By screening for phenotypes in the eggshell ECM, we could take advantage of its complex and highly-ordered 3D structure. The eggshell ECM is predominated by two layers, each of which has a characteristic geometry at cellular and subcellular scales. The inner layer, called the "endochorion", forms a spaced array of dozens of ECM pillars over each epithelial cell. These pillars underlie the "exochorion", which forms a contiguous roof-like layer on top with a pattern of ridges that mirrors the arrangement of cell-cell interfaces of the apposed follicular epithelium. We used sfGFP-tagged versions of protein components from each ECM layer to conduct quantitative image analysis while systematically upregulating and downregulating candidate genes in the tissue to produce a richly dimensional database of co-varying cellular and ECM traits. This allowed us to assess how cell polarity, cell adhesion, actomyosin contractility, and patterned secretion each contribute to many epithelial traits, and how changes in gene activities generate morphological diversity. Taken as a whole, this project has revealed the predominant axes of epithelial and ECM variation in a tissue—across many independent evolutionary events and in-lab genotypes—and enabled us to infer how cell-level mechanisms shape macroevolutionary patterns of tissue morphology.

14 **Modelling the evolution of chemical defense in *Drosophila melanogaster*** Tyler Douglas[1], Poppy Northing[2], David Hill[3], Rebecca Tarvin[2], Richard Fitch[31]Integrative Biology, University of California Berkeley, [2]UC Berkeley, [3]Indiana

State University

While most animals cope with toxin ingestion by breaking down and excreting toxins through metabolic detoxification, some have evolved to do the opposite and uptake/retain toxins for use as chemical defense against predators and parasites. The evolution of chemical defense entails significant change across multiple interrelated trait modules. Toxin sequestration, for instance, may require retuning of the metabolic detoxification pathway to circumvent the breakdown of defensive toxins. Circumvention of toxin breakdown may in turn necessitate modes of resistance that confer resistance without toxin breakdown, such as structural changes in toxin target proteins. Molecular transport systems may become co-opted to facilitate toxin uptake into storage tissues. Additionally, modifications to sensory systems may evolve and overcome the deterrent effects of dietary toxins. How these trait modules co-evolve during chemical defense evolution is unclear. Current understanding is limited both by a lack of genomic data on chemically defended animals and an inability to resolve complex historic interactions between related trait modules. Here, we employ a evolve-and-sequence approach using *Drosophila melanogaster* and the endoparasitoid wasp *Leptopilina heterotoma* to disentangle the evolution of chemical defense with a high degree of genomic and phenotypic resolution. Specifically, we examine whether exposing *D. melanogaster* to endoparasitic wasps and then rearing flies on nicotine-laced (neurotoxic) media can over time select for flies that leverage nicotine for defense against parasitoids. We present phenotypic data on the evolution of toxin resistance, toxin accumulation, and defensive toxin use spanning 20 generations of artificial selection across 40 fly populations (10,000 individuals per population). We also present data on potential resource-based tradeoffs associated with chemical defense evolution by measuring the evolution of active larval metabolic rate, adult body size, and fecundity. Finally, we couple this phenotypic dataset with pooled, full-genome sequencing of evolving populations mid-way through their evolutionary trajectory to characterize the genetic underpinnings of chemical defense.

15 **Balanced Inversions Help Maintain Sexually Antagonistic Polymorphism** Christopher McAllester, John PoolLaboratory 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 as a result 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 across diverse habitats, suggesting the involvement of evolutionary forces beyond local adaptation. We hypothesize that balanced sexually antagonistic selection may be responsible, in accord with the active mate competition among D. melanogaster males and the general 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 many such polymorphic loci only under tight linkage due to recombination load otherwise, 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 of these autosomal inversion arrangements are only transmitted in parents of one sex. We followed with an empirical exploration of selection on inversions between Zambian paternal populations and their embryo and aged adult offspring to detect correlations between inversion status, viability and mating fitness. Results demonstrated non-neutral frequency changes across karyotypes and life history stages, consistent with a complex fitness landscape. Only Inversion 3RK demonstrated a consistent tradeoff between male reproductive success and viability-longevity across genetic backgrounds. This model has implications for sex chromosome evolution, as an autosomal antagonistic haplotype would benefit from linkage to a sex determining locus. Further, balancing selection upon epistatic haplotypes, particularly due to sexually or ecologically antagonistic selection, may contribute significantly to genetic diversity and ongoing evolution and local adaptation in natural populations.

16 **Positioning of the hematopoietic stem cell niche** Kara Nelson, Stephen DiNardoUniversity of Pennsylvania

Niches regulate stem cell behavior and are often found in specific locations within tissues. How that position is established, and whether the reproducible structure/positioning of a niche affects its function is unknown. I am addressing these questions using the hematopoietic niche, the Posterior Signaling Center (PSC). The mature PSC niche signals to hematopoietic progenitors to instruct their maintenance or differentiation into mature hemocytes. The PSC assumes its position 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. Live-imaging of PSC migration revealed that a nearby muscle, visceral mesoderm (vm), moves dorsally alongside the PSC. Thus,

we hypothesized that vm guides PSC positioning. Analysis of mutants that lack vm showed that PSC cells were mis-positioned within the gland, suggesting a requirement for vm in PSC formation. The vm is known to express the secreted ligand, Slit, which is important for morphogenesis of other tissues. To test if Slit could be the vm signal that instructs PSC formation, I analyzed PSC positioning in Slit mutants. These niches were mis-positioned, indicating a requirement for Slit signaling in PSC formation. In future experiments, vm-specific Slit knockdown will reveal if vm is the relevant tissue source of Slit.

17 **Investigating the cellular origins of 3D tissue shapes** Claudia G VasquezBiochemistry, University of Washington

The study of cell shape has taught us many lessons about cellular function; however, we are just beginning to understand how this basic attribute drives form and function at the level of multicellular tissues. The goal of my research program is to uncover the emergent properties that cells use to generate and maintain higher-order tissue structures. The *Drosophila* model system allows for the precise control over molecules and easy access to *in vivo* imaging, and thus is well-suited to uncover the mechanisms that cells use to generate different shapes as the tissue bends, folds, and loops in three-dimensions (3D). I will discuss my past work using these approaches coupled with quantitative image analysis and mathematical modeling to define a mode of lumen formation. In addition, I will describe how my lab will apply these approaches to determine the architectural rules cells use to build 3D tissue structures, with a focus on the developing *Drosophila* renal system (Malpighian tubules). This system involves the generation and extension of tubes that fold on themselves in a stereotypic manner. My laboratory will leverage this system to isolate the parameters that generate specific cell and tissue shapes while keeping the cells in an *in vivo* context. A major challenge in the field is to understand how cells use the same the same starting molecular components, generate and maintain organ structures and functions. How do these properties break down into molecular and physical rules? Which of these rules are broken in disease states? Work in my lab will leverage the known pathways and molecular mechanisms at work in studying cells at an individual basis to uncover how cells integrate these features into highly regulatable 3D tissue forms.

18 **Imaginal disc growth factors regulate epithelial morphogenesis and CO_2 response** Sandra G. Zimmerman[1], Anne E. Sustar[2], Liesl G. Strand[1,3], Celeste A. Berg[11]Genome Sciences, University of Washington, [2]Department of Physiology and Biophysics, University of Washington, [3]Department of Developmental Biology, Stanford University

Drosophila Imaginal disc growth factors (Idgfs) are members of the family 18 glycosyl hydrolases, a group comprising active chitinases and enzymatically inactive chitinase-like proteins (CLPs). A mutation in the enzyme›s catalytic site, conserved in vertebrates and invertebrates, allowed CLPs to evolve independently with functions that do not require chitinase activity. In humans, CLPs function during inflammatory responses, wound healing, and host defense, but when they persist at abnormally high levels at sites of chronic inflammation, such as cancer, asthma, and arthritis, they correlate with disease progression and poor prognoses. Research has focused on the expression patterns of CLPs and their relevance as potential biomarkers of disease or as targets for therapy, but little is known about their physiologic function. We developed tools to characterize the function of Idgfs/CLPs *in vivo* by deleting each of the six *Drosophila Idgf*s (*Idgf1*, *Idgf2*, *Idgf3*, *Idgf4*, *Idgf5*, *Idgf6*) using CRISPR/Cas9. We generated fly lines with single and multiple deletions of *Idgf*s, including a sextuple mutant line lacking all six *Idgf*s. Flies lacking all six *Idgf*s have smaller ovaries and lay fewer eggs. Germ cells form in reduced numbers, and many are lost before reaching the gonad. Larvae have disorganized denticle belts, and adult flies have abdominal cuticle defects and ectopic wing veins. Eggs laid by sextuple mutant females display defects in dorsal-appendage morphology, the frequency and severity of which increase upon exposure to CO_2. Idgfs regulate E-cadherin levels in normoxia and Actin levels in response to CO_2. Given the parallels between *Idgf*s and their human orthologs, our *Idgf* null fly lines provide excellent *in vivo* tools for characterizing *Idgf*/CLP function. By defining the normal molecular mechanisms of CLPs, we will be able to understand the deviations that occur to sway the balance from a physiological to a pathological state.

19 **Tiling mechanisms of the compound eye through geometrical tessellation** Makoto Sato[1], Takashi Hayashi[1], Takamichi Sushida[2], Masakazu Akiyama[3], Shin-Ichiro Ei[41]Institute for Frontier Science Initiative, Kanazawa University, [2]Salesian Polytechnic, [3]Toyama University, [4]Hokkaido University

Tilling patterns are found in many biological structures such as the compound eye and microcolumns in the brain. Among them, hexagonal tilling is dominant probably because it is superior to the other tilling patterns in terms of physical properties such as structural strength, boundary length and space filling.

The *Drosophila* compound eye is made from ommatidial units showing regular hexagonal pattern and is an ideal model

to understand the mechanism of tiling. Interestingly, it also shows tetragonal pattern in some mutant backgrounds. Here, we propose a universal mechanism of ommatidial tilling. Voronoi diagram is often used to equally divides multiple areas according to the distance from the center of each area. We found that the wildtype hexagonal pattern and mutant tetragonal pattern perfectly fit with Voronoi diagram. Incorporating the tissue-wide tension along the dorsal-ventral axis observed *in vivo*, the hexagonal pattern is transformed to the tetragonal pattern.

How does ommatidial shape obey the geometrical Voronoi patterns? To answer this question, we focused on mutant eyes, in which the tilling pattern becomes stochastic. Surprisingly, such a stochastic ommatidial pattern also fit with Voronoi diagram except for occasional mismatching found in smaller and larger ommatidia. The growth of ommatidia, which is largely affected by the number of cells within individual ommatidia, may play critical roles. We therefore incorporated the differential growth of ommatidia into Voronoi diagram. Compared with standard Voronoi diagram, we found that weighted Voronoi diagram, in which the concentric growth rate is proportional to the ommatidial size, nicely fit with the stochastic mutant pattern. Thus, physical stretch of the eye tissue and geometrical tessellation through the concentric growth of ommatidia co-operatively determine the ommatidial tiling patterns.

20 **The Role of *crossveinless-c (cv-c)* in Caudal Visceral Mesoderm (CVM) Migration** Jayden Ogbodo, Afshan IsmatBiology, University of St. Thomas

Regulation of actin polymerization occurs mainly through Rho family GTPases, of which there are three main members: Rho, Rac, and Cdc42. GTPases are regulated themselves by two types of proteins: GEFs (Guanine Exchange Factors) that convert a GTPases from an inactive to active state, or GAPs (GTPases-Activating Proteins) that inactivate GTPases. The focus of this project is on the gene *crossveinless-c* (*cv-c*), which encodes a RhoGAP protein, a GAP specific for the GTPase Rho. A previous study showed that *cv-c* mRNA was expressed in several migratory cell types in the embryo, including the caudal visceral mesoderm (CVM). The absence of *cv-c* resulted in delayed and less orderly dorsal closure by leading edge cells and an almost complete lack of migration of Malpighian tubules. In both cases, the defect in cell migration was the result of problems in actin polymerization and organization, a role that clearly fits with the function of a RhoGAP. The CVM cells migrate as a loose collective along the entire length of the embryo. Using *croc-lacZ*, a CVM cell-specific reporter, we show that, in the absence of *cv-c*, CVM cells mis-migrate. We are also in the process of using a myristoylated-GFP in the *cv-c* mutant to visualize cell protrusions and possible changes in the actin cytoskeleton. We are also currently trying to see if there is a genetic interaction between *cv-c* and Rho in CVM migration.

21 **Lessening cell-ECM adhesion: a kick off point triggering basal epithelial folding.** Maria Martin-Bermudo[1], Andrea Valencia[2], Nargess Khalilgharibi[3], Yanlan Mao[31]CABD, [2]CSIC, [3]. MRC Laboratory for Molecular Cell Biology. University College of London

Apical and basal tissue folding is essential for shaping the complex three-dimensional structures of organs. However, while apical folding has been extensively studied, little is known about the mechanisms underlying basal folding. In addition, although previous studies have identified local changes in actomyosin activity and cell adhesion as key regulators of tissue folding, how these changes are coordinated in time and space remains poorly understood. Here, we use the primordium of the wing, the *Drosophila* wing imaginal disc epithelium, to analyse the role of cell-basement membrane (BM) adhesion, mediated by integrins, on the regulation of basal tissue folding. The wing imaginal disc is an epithelial sac that contacts on its basal side with a BM. During its development, the disc folds basally along a row of cells, the future wing margin cells, to give rise to the bilayer adult wing. We show that basal disc folding involves four interconnected events: reduction of integrin levels, detachment from the BM, basolateral actomyosin accumulation and reorganization and cell shortening. Furthermore, we find that maintenance of high integrin levels in wing margin cells prevents these changes, leading to abnormal disc folding. Contrariwise, reduction of integrin levels in an ectopic location recapitulates the events that take place at the wing margin, resulting in the formation of an ectopic fold. In addition, computational exploration, shows that the reduction of adhesion to the BM must precede changes in F-actin reorganization and cell shortening for proper basal epithelium bending. Finally, we find that downregulation of integrin levels triggers a basolateral shift of E-cadherens localization. Based on these results, we propose that basal folding is triggered by a switch from high cell-ECM adhesion to cell-cell adhesion on the basolateral side of cells, which in turn leads to reorganization of actomyosin activity and cell shape changes.

22 **Effects of maternal BMP signaling in *Drosophila melanogaster* early embryonic development** Daniel Bressan de Andrade, Marcio Fontenele, Helena AraujoInstitute of Biomedical Sciences, Federal University of Rio de Janeiro

The Bone Morphogenetic Proteins (BMP) pathway is present in every animal group studied to date and plays several roles in development. One of the main functions of this pathway is to establish embryonic territories along the dorsal-ventral axis. In *Drosophila melanogaster* the main BMP ligand, *decapentaplegic* (*dpp)*, has two distinct roles on early embryogenesis: a well-described zygotic effect on the patterning of dorsal territories, and a maternal effect throughout the embryo, whose mechanism of action remains to be elucidated. In this work, we analyzed the effects on early development of impairing the reception of the maternal BMP signal through two different methods: germline clones of null mutants for the BMP receptor *thickveins* (*tkv*), which leads to total loss of *tkv* on the germline and, consequently, no maternal deposition of *tkv* mRNA or protein product; and *tkv* RNA inteference (RNAi) directed to maternal germline through the GAL4-UAS system, which leads to *tkv* mRNA inhibition and degradation in the oocyte. Both experiments showed previously unknown functions for the BMP pathway on early development, with defects on the coordination of mitotic divisions and differentiation of the pole cells, which correspond to the primordial germ cells. Furthermore, we confirmed the effect previously described on the dorsal-ventral axis, through a modulation of the Dorsal protein nuclear translocation gradient, a well established mechanism for DV patterning. Then we asked if this inhibition of the maternal BMP signaling could also affect the maternal-to-zygotic transition (MZT), which consist on the intertwined processes of maternally deposited mRNAs degradation and zygotic genome activation. To test that, we performed qRT-PCR for genes with a central role on MZT, including *pan gu*, *smaug* and *zelda*, on both wildtype and *tkv* RNAi embryos, in two distinct time points: at the beginning (0-1h) and on an advanced phase of MZT (2-3h). We found that maternal BMP signal inhibition has little effect on the earlier stage, but result in relevant changes on the mRNA levels for theses genes on the latter, in a way that suggests a delay or loss of coordination on the MZT process. This work contributes to the expansion of our knowledge about the factors that dictate the first steps on the formation of a new organism, showing that a maternal signal from the BMP pathway is essential for processes such as cleavage, germline differentiation and the regulation of gene expression.

23 **The *Drosophila* tracheal system controls sex differences in gut shape** Laura Blackie[1,2], Pedro Gaspar[1], Salem Al Mosleh[3], Todd Schoborg[4], Boxuan Cao[2], Yuhong Jin[5], Agata Zielinska[5], Elif Elveren[6], Nursu Cakir[7], Marta Varela[2], Lakshminarayanan Mahadevan[3], Irene Miguel-Aliaga[1,21]MRC London Institute of Medical Sciences, [2]Imperial College, [3]Harvard University, [4]University of Wyoming, [5]Francis Crick Institute, [6]Baskent University, [7]Near East University Hospital

The shape of organs in the body and how they are positioned relative to each other has implications for organ function and local inter-organ communication. For example, if a gut loop becomes twisted on itself forming a volvulus, passage of food is disrupted and the gut cannot function properly. Furthermore, our work in *Drosophila* has revealed that an organ's shape can additionally influence its communication with neighbouring organs. Indeed, the close proximity of the testes and the gut facilitates local signalling and exchange of metabolites between these organs, sustaining reproductive output. This raises the question of whether the shape and position of organs is stereotypical and, if so, how this organ and inter-organ geometry is established and maintained. We have embarked on a comprehensive characterisation of organ shape and position using micro-computed tomography to image whole *Drosophila* adults. We have used image segmentation and 3D shape analysis to comprehensively map 3D shape and the 3D relationships between neighbouring organs. We find that both the coiling of the long gut tube and its contact with other organs is non-random, stereotypical and sexually dimorphic. We identify the tracheal system as playing a supportive structural role in maintaining gut shape in the adult. In response to Branchless-Breathless signalling from the gut muscle, the tracheal system is recruited to the gut surface in a sexually dimorphic pattern. The trachea then hold the gut coils into position. Through this work, we shed light on the previously under-appreciated third dimension of inter-organ relationships.

24 **Maternal metabolic stress regulates intestinal stem cell differentiation by metabolite inheritance** Helin Hocaoglu, Leah Eller, Farhan Abu, Benjamin Ohlstein, Matthew SieberUT Southwestern Medical Center

Maternal diet and metabolic stress have a profound effect on health and disease susceptibility of progeny. Previous studies examining this heritable effect have focused on gestational stress exposure which occurs during pregnancy, and these stresses are shown to be transferred into the progeny through placenta. However, mechanisms of non-gestational stress, which happens through exposure before pregnancy, remain elusive. In this study, to overcome the challenge of dissecting the differences between non-gestational and gestational stress, we used the oviparous Drosophila system, which lack a placental connection, and we examined how metabolic stress can be inherited through the germ cells. To uncover this relationship between non-gestational maternal stress and progeny health, we inhibited insulin signaling, the primary regulator of oogenesis, as a tool to disrupt maternal germ cell metabolism. By manipulating insulin signaling to mimic maternal metabolic stress, we found that mature oocytes from stressed mothers have low levels of NAD^+. NAD^+ deficiency in oocytes caused embryos and adult progeny to have decreased methionine cycle intermediates. Lower

levels of SAM, which is a methionine cycle intermediate, caused de-repression of 550 genes during embryogenesis most of which were intestinal genes involved in lipid digestion, uptake, storage, protein digestion and nutrient transport. In parallel to this transcriptional response, we showed that reduced methionine cycle activity alters intestinal stem cell lineage differentiation leading to a significantly increased proliferation and an elevation in enteroendocrine cell frequency. We have found that these intestinal phenotypes are due to impaired delta-notch signaling which is known to regulate intestinal stem cell differentiation and proliferation. Intriguingly, we showed that maternal NAD^+ supplementation or progeny methionine supplementation can rescue these intestinal phenotypes. Overall, our data suggest that maternal metabolic stress controls intestinal stem cell lineage through methionine cycle by regulating delta-notch signaling.

25 **Integrated Stress Response signaling in adipose tissue acts as a systemic regulator of reproduction** Lydia Grmai, Manuel Michaca, Emily Lackner, Deepika VasudevanCell Biology, University of Pittsburgh

Reproduction is a systemic decision that relies on nutrient availability to support the high energetic cost of gametogenesis and reproductive behaviors. Disorders that yield insufficient/excess body fat mass are associated with decreased ovulation and infertility. However, the cellular mechanisms that relay nutrient status to alter reproductive output are not well understood. We have identified the Integrated Stress Response (ISR) as a fat tissue metabolic sensor that regulates both oogenesis and ovulation via interorgan signaling. The ISR pathway, which induces expression of the transcription factor Atf4, exhibits basal levels of activation in the fat body, a highly metabolic and adipose-rich tissue. The fat body supports oogenesis by synthesizing and trafficking yolk proteins to maturing oocytes, and impairing yolk uptake by oocytes is known to disrupt oogenesis. We found that loss of *Atf4* in the fat body results in oogenesis arrest and follicle death. Interestingly, this was accompanied both by decreased yolk protein abundance in the fat body and decreased yolk granule abundance in oocytes. Yolk granules are trafficked to maturing oocytes as lipoprotein particles, assemblies of yolk with lipid droplets. We found that depleting *Atf4* in the fat body impaired lipid droplet formation and decreased mRNA levels of the lipase *brummer* (*bmm*). We are currently investigating a broader role for *Atf4* in lipoprotein synthesis and assembly.

To determine the ISR signaling components upstream of *Atf4* in the fat body, we depleted ISR kinases *perk* or *gcn2* in the fat body. Loss of either kinase resulted in modest follicle death and joint depletion of both *perk* and *gcn2* resulted in oogenesis arrest to a similar degree as depletion of *Atf4.* Interestingly, animals lacking *perk* or *gcn2* also exhibited retention of excess oocytes in the ovary. This “egg retention” is symptomatic of defects in ovulation, which is regulated by subsets of sexually dimorphic neurons. Through a selective screen, we identified a neuropeptide, CNMamide (CNMa), in the fat body that promotes egg-laying behavior. Our preliminary data indicate that CNMa expression is Atf4-dependent, and depletion of *CNMa* in the fat body led to egg retention. Strikingly, restoring CNMa expression prevented the egg retention phenotypes caused by depletion of *perk* or *gcn2* in the fat body. Taken together, our results implicate the Integrated Stress Response as a metabolic sensor in fat body that informs female reproduction in at least two ways: by promoting ovulation via yolk granule synthesis, and ovulation via interorgan signaling.

26 **Lactate and glycerol-3-phosphate metabolism cooperatively regulate larval growth in a tissue nonautonomous manner** Madhulika Rai[1], Shefali Shefali[2], Sarah Carter[3], Hongde Li[4], Maria C. Sterrett[3], Geetanjali Chawla[5], Jason M Tennessen[31]Biology, Indiana University Bloomington, [2]Biology, Indian University Bloomington, [3]Indiana University Bloomington, [4]UTSouthwestern, [5]RNA Biology Laboratory, Regional Centre for Biotechnology, NCR Biotech Science Cluster

Drosophila larval development requires the rapid conversion of nutrients into biomass. In response to these biosynthetic demands, larval metabolism exhibits the hallmark features of aerobic glycolysis, a metabolic program ideally suited to synthesize macromolecules from carbohydrates. Previous studies from our lab have demonstrated that the enzymes Lactate Dehydrogenase (LDH) and Glycerol-3-phosphate dehydrogenase (GPDH1) are essential for cooperatively maintaining the larval glycolytic program. Although disruption of either enzyme has minimal effect on larval growth, simultaneous loss of both enzymes results in a synthetic lethality and aberrant carbohydrate metabolism. These findings, however, are based on studying loss of *Ldh* and *Gpdh1* in the whole body and raise the question of how these two enzymes coordinate larval metabolism across multiple tissues. To address this question, we have used RNAi to determine how tissue-specific depletion of *Ldh* and *Gpdh1* affects larval growth and metabolism. Our results demonstrate that loss of *Ldh* within either the fat body or muscle of *Gpdh1* mutants lead to systemic growth defects in larvae. Hence, *Ldh* and *Gpdh1* can influence larval growth and metabolism in a cell nonautonomous manner, indicating that the cooperative activity of these two enzymes within individual tissues can induce systemic signals that coordinate intercellular metabolic

states with growth of the entire organism. To find these systemic signals, we performed transcriptomic analysis and interestingly, found that the cytokine UPD3 gets upregulated in response to changes in LDH and GPDH1 activity. Further, loss of *Upd3* in *Ldh; Gpdh1* double mutant larvae showed milder growth defects in comparison to *Ldh; Gpdh1* double mutant. This suggests that *Upd3* is a key systemic signal coordinating whole larval growth with tissue-specific levels of LDH and GPDH1. Overall, our findings hint at a mechanism that coordinates larval growth with the rate of glycolytic flux in individual tissues.

27 **Sphingolipid control of neural circuits by glial catabolism** Emma Theisen[1], John Vaughen[1], Irma Magaly Rivas-Serna[2], Tom Clandinin[2], Tom Clandinin[11]Stanford University, [2]University of Alberta

Brain circuit assembly, synaptic function, and structural plasticity are underpinned by dynamic membrane rearrangements. Though proteins and cytoskeletal forces have been extensively studied during membrane remodeling, how lipids contribute to membrane dynamics is underexplored. Developing and adult brains are enriched with sphingolipids, a lipid class strongly linked to neurological diseases yet whose *in vivo* functions remain enigmatic. Here, by genetic dissection of enzymes controlling the balance of sphingolipid anabolism and catabolism, we show that glia non-autonomously degrade neuronal sphingolipids to sculpt morphology. Specifically, glial subsets produce Glucocerebrosidase (GBA) and acid-sphingomyelinase (aSMase) for sphingolipid catabolism, and glial knockout of these enzymes causes neuronal lysosomal enlargement. We further demonstrate that glial *GBA* knockout triggers diurnal ubiquitinated aggregate formation, sleep loss, and also abrogates neurite remodeling in a circadian circuit that dynamically grows and shrinks every day. Daily neurite remodeling is governed by the balance of sphingolipid biosynthesis and degradation, and specific sphingolipids fluctuate diurnally in the brain, unlike most membrane phospholipids.

Remarkably, these circadian sphingolipids are also induced during brain development, alongside a unique sphingolipid subset that is selectively enriched during neural circuit assembly. Notably, these developmental sphingolipids represent a large fraction of the adult lipids accumulating in *GBA* or *aSMase* mutants. Consistent with developmental origins for adult mutant phenotypes, the aberrant lysosomes in *GBA* and *aSMase* perturbations begin during pupal brain development. By integrating our lipidomics datasets with existing scRNA-sequencing datasets, we find that the dynamic developmental sphingolipidome is controlled by synchronized neuronal biosynthesis and glial catabolism. During adult brain synaptogenesis, all neurons transiently induce *lace*, the rate-limiting enzyme for sphingolipid biosynthesis. What is the function for these developmental sphingolipids? Preliminarily, we find that impaired sphingolipid catabolism causes misrouting of synaptic active zone proteins, whereas impaired sphingolipid metabolism causes widespread neural targeting defects and ubiquitinated aggregates. Thus, the striking compartmentalization of sphingolipid metabolism between glia and neurons likely acts to finetune neuronal structure and function during both development, adult circuit remodeling, and disease.

28 **Myoinhibiting peptide precursor affects the diet-gut-brain axis and leads to neurodegeneration** Francesca Di Cara, Stephanie Makdissi, Rihab Loudhaief, Eden BishopMicrobiology and Immunology, Dalhousie University

Neuroinflammation is a common feature of the pathogenic mechanisms involved in various neurodegenerative diseases (NDs). NDs are a leading cause of global death and disability, and the incidences are expected to increase in the following decades if prevention strategies and successful treatment remain elusive. To date, the etiology of NDs is unclear due to the complexity of the mechanisms of diseases involving genetic and environmental factors. The gastrointestinal tract communicates with the nervous system through a bidirectional network of signaling pathways called the gut-brain axis. Alteration of communications in the gut-brain axis is emerging as an overlooked cause of neuroinflammation. To dissect the gut-brain axis in the context of NDs, we generated two *Drosophila* mutant strains presenting defects in mitochondrial metabolism and peroxisomal metabolism, respectively in the enterocytes to creat metabolic stress. Metabolomic and lipidomics analyses revealed defects in ceramide metabolism in the guts of both mutants. Transcriptomic and genetic analyses revealed that this metabolic defect leads to the induction of a battery of neuropeptides from enteroendocrine cells. Genetic studies showed that BMP-mediated induction of the neuropeptide Myoinhibiting peptide precursor (Mip) in the guts of both mutants leads to neuroinflammation and neurodegenerations of dopaminergic neurons and ultimately causes behavior defects. Induction of Mip from the gut and consequent neurodegeneration and behavior deficits can be recapitulated in wildtype flies fed a defined high-fat diet and in flies expressing their guts a human mutant variant of *α-synuclein* linked to the onset of Parkinson Disease. Conversely, treatment of the mutant flies with niacin, a lipid scavenger, rescues, at least in part, the neurodegeneration defects.

This work demonstrated that lipotoxic stress triggers expression of neuropeptides that impact the gut-brain communication and may cause neurodegeneration. Our work advances the understanding of the impact of metabolism in gut-brain axis communication and its link to neurodegeneration.

29 ***Myc* mediates the scaling of RNA and proteins with ploidy in the *Drosophila* midgut** Christopher Amourda[1], Tomotsune Ameku[2], Laura Martin-Coll[2], Dafni Hadjieconomou[2], Santiago Vernia[2], Irene Miguel-Aliaga[21]MRC-LMS, Imperial College London, [2]Imperial College London

In recent years, it has become apparent that non-gonadal organs differ between the sexes, and that these somatic sex differences contribute to sex-specific or sex-biased physiology such as reproduction. We have used the *Drosophila* intestine to investigate the nature and significance of somatic sex differences. We have recently found that *Drosophila* midguts are highly sexually dimorphic at DNA (i.e. ploidy), RNA and protein levels. Interestingly, sex differences in the proteome are only weakly correlated to sex differences in the transcriptome, and ribosomes and other translation related genes are female-biased at both transcript and protein levels, suggestive of sex differences in post-transcriptional mechanisms. We identify the gene *Myc,* known to control several aspects of transcription, translation and endoreplication, as being female-biased in fly midgut. Genetic manipulations of Myc expression reveal a role for Myc in regulating sex differences in endoreplication and, more unexpectedly, in scaling the amount of RNA and protein with the ploidy of the cell. Our results therefore reveal previously unrecognised layers of sex-biased regulation of gene expression that contribute to sex differences in organ physiology.

30 **Aging Fly Cell Atlas** Tzu-Chiao Lu[1,2], Maria Brbić[3,4,5], Heinrich Jasper[6], Stephen R. Quake[4,7,8], Hongjie Li[1,21]Huffington Center on Aging, Baylor College of Medicine, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, [3]Swiss Federal Institute of Technology, [4]Chan Zuckerberg Biohub, [5]Department of Computer Science, Stanford University, [6]Immunology Discovery, Genentech, [7]Department of Bioengineering, Stanford University, [8]Department of Applied Physics, Stanford University

Aging is accompanied by functional decline of all tissues, but it is still largely unknown how aging impacts different tissues in a cell type-specific manner. Here, we present the Aging Fly Cell Atlas (AFCA) that profile single-nucleus transcriptomes of the whole Drosophila from four different ages with both male and female. 162 distinct cell types are characterized, and different aging features, including changes of cell composition, number of differentially expressed genes, number of expressed genes per cell, transcriptome noise, and cell identity, are analyzed and compared between cell types. We also develop an aging clock model to identify cell types and genes that better predict the fly age, and ribosomal proteins genes are identified as conserved aging clock factors across diverse species. By combing all aging features, adipose cell types show the highest aging score, followed by two reproductive systems. Our data provide a valuable resource for studying the fundamental principles of aging in complex organism.

31 **Vitamin A deficiency triggers a novel transmembrane protein that stabilizes degenerating photoreceptors.** Deepshe Dewett[1], Khanh Lam-Kamath[1], Ethan Wagner[1], Mukesh Kumar[2], Andrej Shevchenko[3], Maryam Lab[4], Kourosh Zarringhalam[4], Jens Rister[41]Biology, UMASS Boston, [2]Harvard med school, [3]Max plank institute, [4]UMASS Boston

Vitamin A is essential for vision and must be obtained through our diet. Vitamin A deprivation (VAD) severely disrupts the morphology of the photoreceptors, causes a loss of the light-sensing pigments, and is the leading cause of preventable childhood blindness. However, even chronic VAD in *Drosophila* does not lead to photoreceptor death, which led us to hypothesize that an unknow mechanism stabilizes them to preserve visual function. To elucidate the underlying molecular mechanism, we employed RNA-seq and mass-spec to identify differentially expressed genes and proteins, respectively, in flies raised with or without vitamin A. We identified factors that belong to the categories such as phototransduction, synthesis of the retinal chromophore, aminoacyl-tRNA synthetases, major nutrient reservoir proteins, calcium buffers, and stress or immune responses. Strikingly, we identified the novel transmembrane protein Mps as the most upregulated protein in response to VAD (~140-fold) and the corresponding gene showed the second-strongest transcriptional increase.

To investigate Mps's function, we generated an Mps antibody and found that it is expressed in the damaged light-sensing rhabdomeres of the vitamin A-deficient photoreceptors. Moreover, the rhabdomere membranes of vitamin A deficient *mps* mutants are collapsed and show abnormal, curtain-like invaginations. To understand the molecular mechanism by which Mps stabilizes the photoreceptors, we sought to identify its binding partners with a yeast-2-hybrid assay. We identified the major photoreceptor scaffolding protein, InaD, as a potential interaction partner of Mps. We performed *in vitro* studies where we transfected S2 cells with tagged Mps and InaD plasmids under the control of an actin promoter.

Immunohistochemistry studies showed that Mps and InaD colocalize to the cell membrane. Moreover, co-ip analyzes showed pull down of InaD with Mps after cotransfection in S2 cells.

In conclusion, our data suggest that the novel transmembrane protein Mps stabilizes damaged vitamin A-deficient photoreceptors by interacting with the major scaffolding protein InaD. These results give mechanistic insights into how the eye responds to environmental stresses and could inspire novel therapeutic approaches to treat human eye diseases.

32 **Origin and structural evolution of de novo genes in *Drosophila*** Junhui Peng, Li ZhaoRockefeller University

Understanding how genes originate and evolve is crucial to explaining the origin and evolution of novel phenotypes and, ultimately, the diversity of life. *De novo* gene origination from nongenic sequences has been proved as a relatively common mechanism for gene innovation in many species and taxa. These young proteins provide a unique set of candidates to study the structural and functional origination of proteins. Despite extensive efforts to identify *de novo* genes in different species, our understanding of their protein structures and how they originate and evolve are still very limited.

In this study, we applied highly accurate reference-free progressive whole genome alignments and identified hundreds of *de novo* gene candidates in *D. melanogaster* that originated within the *Drosophilinae* lineage. We found a gradual shift in sequence composition, evolutionary rates, and expression patterns with their gene ages, indicating possible gradual shifts of their functions. Interestingly, we found little overall changes in structural properties (structural disorder and probability of being transmembrane proteins) for proteins encoded by *de novo* genes along their evolutionary trajectories. Using single-cell RNA-seq data in testis, a hotspot for *de novo* gene origination, we found that several young *de novo* genes had biased expression in the early spermatogenesis stage, indicating unneglectable roles of early germline cells in the *de novo* gene origination in testis.

We further combined AlphaFold2 prediction and molecular dynamics simulations to study the 3D structures and structural evolution of the identified *de novo* gene candidates. Our results suggest that while many of these candidates are highly disordered, a small subset may be well-folded. Most of the potentially well-folded *de novo* gene candidates adopt known structural folds, but some may have novel structural folds. By using ancestral sequence reconstruction and structural modeling, we found that these potentially well-folded proteins are often born folded. We observed one case where disordered ancestral proteins become ordered within a relatively short evolutionary time frame, suggesting that even for evolutionarily young genes, protein structures evolve slowly. Altogether, we provide a systematic overview of the origination, evolution, and structural changes of *Drosophilinae*-specific *de novo* genes.

33 **Origination and evolution of transfer RNA genes in Drosophila** Dylan Sosa[1], Manyuan Long[21]ecology & Evolution, University of Chicago, [2]Ecology & Evolution, University of Chicago

Transfer RNA (tRNA) are ubiquitous and essential molecules that carry amino acids corresponding to messenger RNA (mRNA) codons to facilitate protein synthesis. This primary function of tRNA as an adaptor between amino acids and the genetic code is well studied. However, questions on the origination and evolution of tRNA remain outstanding. Whether the genes encoding such a critical molecule continue to be evolved, how and when they originate, and how they are utilized by newly evolved protein-coding genes are unknown and present important problems whose study will improve our understanding of gene origination and molecular evolution.

We annotate and compare the diversity of nuclear-encoded tRNA genes in 15 *Drosophila* species to understand tRNA evolution and origination. Using these annotations we perform whole genome alignment of the 15 Drosophila species' genome assemblies to conduct gene age dating of D. melanogaster tRNA genes. Of the 291 age-dated, chromosomal-encoded tRNA genes we find that 3 are species-specific, having no homologous or syntenic relationship with tRNA genes of the other 14 species. We further investigate the evolutionary history of tRNA genes by analyzing their duplication mechanisms and the gain and loss of tRNA genes across the *Drosophila* phylogeny.

We use the annotations resulting from these analyses to approach the question of how evolutionarily young genes interact with the D. melanogaster genomic tRNA pool. We hypothesize that new genes, especially those of putative *de novo* origination, would be less adapted to existing translational machinery and thus would be less optimized in their tRNA usage. To test this we compute tRNA adaptation index values for young and ancient D. melanogaster protein coding genes and compare them to detect the strength of translational selection on genes of different ages, origination mechanisms, and phylogenetic groups. We observe that indeed young genes, especially young orphan genes and

melanogaster subgroup-specific genes, are less biased in their codon usage indicating that they are not optimally using the available tRNA pool. Based on these preliminary data, we plan to functionally investigate the three melanogaster-specific tRNAs and we will be conducting tRNA-seq experiments in seven Drosophila species to characterize their abundance, identify tissue-specific expression, and determine any relationship between codon usage and tissue specificity.

34 **The genetic basis of neural circuit evolution for *Drosophila* mate preferences** Emily L Behrman, David SternHHMI Janelia Research Campus

Behaviors that arbitrate interspecies courtship are at the crux of evolutionary barriers between species. Species- and sex-specific pheromone profiles 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 *D. melanogaster* males, but the taste inhibits courtship in males of their close relative, *D. simulans*, whose females do not produce that pheromone. Males of both species utilize the same gustatory receptors to taste the female *D. melanogaster* pheromone, but the signal is then differently propagated to the central courtship-driving neurons in the brain. This results in net activation of the central courtship-driving neurons in *D. melanogaster* and net inhibition of those same neurons in *D. simulans*. We identify a single gene that has evolved to reshape this neural circuit and change the male's response to the pheromone 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. We provide a unique mechanistic understanding of how behavior evolves starting with expression of a gene in specific neurons to alternations in the neural circuit to change behavior.

35 **Development of fast motion detectors in insect eyes: the example of the male housefly small target motion detector** Antoine Donati[1], Yunchong A Zhao[2], Eleanor Terner[2], Michael Perry[21]University of California, San Diego, [2]UCSD

Many insect species have evolved the ability to detect and chase rapidly moving prey or mates, and such behavior often corresponds to the presence of a specialized eye region. The best-studied example is the "small target motion detector" (STMD) of the dorsal-anterior retina of the male housefly (*Musca domestica*), which allows them to perform highly skilled female pursuit in full flight. We use this feature of the male housefly eye to gain insight into how new traits and cell types appear during development and evolution. We first used single cell sequencing (scRNAseq) to identify differentially expressed genes in male-specific cell types during pupal retina development. We next used CRISPR targeted gene knockout, and find that the transcription factors Doublesex (Dsx) and Spineless (Ss) interact in a positive feedback loop that is critical for the specification of a new photoreceptor type within the STMD: a photoreceptor in the R7 position. This novel type of neuron expresses broad-spectrum Rhodopsin 1 instead of color-sensitive Rhodopsins, and projects to the lamina neuropil instead of the medulla. Dsx and Ss together downregulate the transcription factor Spalt and upregulate the transcription factor Dve, which are both known to regulate Rhodopsin expression in *Drosophila melanogaster*.

We are now using piggyBac transgenesis to test whether the rest of the eye can be converted to this new fate by expression of Dsx alone or if this requires another factor specifically expressed in the dorsal-anterior eye. It will be interesting to use this system to understand how this new gene regulatory network evolved by looking at Dsx binding sites near target genes, finding the enhancers that drive Dsx expression in the dorsal-anterior eye, and by looking at motion detectors in the eyes of other insect species such as Hoverflies.

36 ***Sox21b* contributes to the rapid diversification of a novel male genital structure between *Drosophila simulans* and *Drosophila mauritiana*** Amber M Ridgway[1], Javier Figueras Jimenez[2], Joanna F D Hagen[3], Emily Hood[1], Maria D S Nunes[1], Alistair P McGregor[21]Biological and Medical Sciences, Oxford Brookes University, [2]Durham University, [3]Johns Hopkins University

Drosophila simulans and *D. mauritiana* exhibit striking morphological differences in the size, shape, and bristle composition of the male periphallic genitalia, despite only diverging 240,000 years ago. This raises the question of what genetic differences have led to this rapid phenotypic divergence, however, the underlying developmental programme required to form these structures is still not well understood. To address this, we generated RNA-Seq data from developing genitalia of *D. simulans* and *D. mauritiana* males and focused on a subset of differentially expressed transcription factors (TFs) between these species, to pinpoint candidates that could be both developmentally, and evolutionary important.

We found that *Sox21b* is more highly expressed in *D. mauritiana* than *D. simulans*, and is located within a region on chromosomal arm 3L known to contribute to the posterior lobe (PL) size difference between these two species. RNAi knockdown of *Sox21b* in *D. melanogaster* resulted in larger PLs consistent with the species difference, revealing that this TF represses PL growth. To determine whether *Sox21b* has evolved between the two species, we generated reciprocal hemizygotes that differed only by which species the functional allele of *Sox21b* was from. Flies with functional *D. mauritiana Sox21b* had smaller PLs than those with the *D. simulans* allele. In addition, the *D. mauritania Sox21b* allele affected the shape of the lobe. This confirmed that *Sox21b* has contributed to the evolution of PL morphology between these two species.

To understand how *Sox21b* has evolved, we then screened the activity of putative enhancers within the *Sox21b* locus of *D. melanogaster*, to uncover the source of its differential expression between species. We identified an 800 base pair region upstream of the gene that drives expression at the base and ventral region of the PL. Sequence analysis suggests this element is regulated by Abdominal B which may repress *Sox21b* to promote PL growth. We are currently comparing the activity of this enhancer among species and investigating the consequences of *Sox21b* differences on copulation.

37 **Rapid functional divergence of *Drosophila* protamines hints at their roles in suppressing genetic conflicts between sex chromosomes** Ching-Ho Chang[1], Isabel Mejia Natividad[1], Harmit S Malik[1,21]Division of Basic Sciences, Fred Hutchinson Cancer Center, [2]Howard Hughes Medical Institute, Fred Hutchinson Cancer Center

Most eukaryotes deploy histones for genome packaging functions. However, many animal species accomplish tighter packaging of genomes in sperm using short, positively charged proteins, called protamines. Whereas histones are ancient and highly conserved, protamine repertoires have independent evolutionary origins, differ dramatically across different animals, and evolve rapidly in mammalian lineages. The rapid evolution and positive selection of protamine genes indicate they might have functions other than condensing sperm DNA. Our previous phylogenomic studies in *Drosophila* showed that the only conserved putative protamine, *CG30056*, is dispensable for male fertility, whereas two young, rapidly evolving protamine genes, *Prtl99C* and *Mst77F*, are essential for male fertility in *D. melanogaster*. Based on the transcriptional silencing role of protamines, we further propose some protamines can suppress sex chromosome-linked meiotic drivers, which kill sperm without themselves to bias their own transmission. To test this hypothesis and reveal the selection force driving their evolution, we genetically dissected *Prtl99C* and *Mst77F* in *D. melanogaster*. We showed that replacing either gene with its ortholog from related species, *e.g.*, *D. yakuba* and *D. ananassae,* is sterile or subfertile, indicating the rapid functional divergence of protamines. The replacement flies of either protamine gene have a proper expression of the replaced protamine in their sperm head prior to the individualization, a process removing most sperm cytosol and separating individual sperm, but not in mature sperm. This result suggests the presence of protamines in mature sperm is critical for their function. We also conducted transgenic and knockout experiments in *D. yakuba*, and surprisingly found *Prtl99C* is not essential in *D. yakuba*, despite expressing in mature sperm. These findings conclude Prtl99C first acquired their expression in mature sperm and recently evolved essential functions in the *D. melanogaster* lineage. In contrast, some *Mst77F* replacement flies produce sex-biased offspring, providing vital support to our genetic conflict hypothesis. Altogether, our functional analyses on *Drosophila* essential protamines reveal their rapid functional divergence and further hint at their roles in sperm, particularly in suppressing genetic conflicts between sex chromosomes.

38 **Function and regulation of amyloids in developing *Drosophila* embryo** Kuan-Chih Peng[1], Jaime Carrasco[2], Zelha Nil[3,4], Paulo Leal[4], Javier Oroz[2], Rubén Hervás[5], Kausik Si[41]Si Lab, Stowers Institute for Medical Research, [2]Rocasolano Institute of Physical Chemistry, [3]Department of Molecular and Human Genetics, Baylor College of Medicine, [4]Stowers Institute for Medical Research, [5]University of Hong Kong

Amyloids are generally believed to form from misfolded or unfolded proteins causing diseases. However, emerging evidence indicates that amyloids could serve biological functions in a regulated way. Herzog (Hzg), a membrane-associated phosphatase, is one such example, which controls embryonic patterning in *Drosophila melanogaster*. At the onset of gastrulation an amyloid-like assembly confers enzymatic activity to Hzg. Defined biochemical activity, and developmental stage-specific amyloid formation, provide an opportunity to ask what are the phenotypic consequences of Hzg amyloid formation, how amyloid formation activates the enzymatic function, and how cells control the formation and dissolution of a amyloid. We have generated several Hzg variants that either blocks Hzg assembly or change the properties of the assembled state. Our findings indicate that amyloidogenic assembly of Hzg is important for proper embryonic development. Using NMR we have analyzed the structure of catalytically inactive Hzg monomer. It appears that in monomeric Herzog, the amyloid forming domain blocks access to the catalytic site, suggesting amyloid formation

may expose the catalytic domain. Finally, to uncover how Hzg amyloid is regulated, we have developed reporters that scores Hzg self-assembly. We anticipate studying amyloid as part of normal biology, as opposed to an unintended consequence, would help us understand why amyloid forming proteins are prevalent in all branches of life.

39 **Endocytic regulation of the spatial organization of Dachsous-Fat signaling** Artem Gridnev, Jyoti R MisraDepartment of Biological Sciences, University of Texas at Dallas

Stereotypic dimensions of organisms and their organs result from precise coordination of growth and morphogenesis during development, and the evolutionarily conserved protocadherins Dachsous and Fat play a critical role in coordinating these processes, where they regulate growth by influencing the Hippo pathway, and morphogenesis by influencing planar cell polarity (PCP) respectively. However, there are critical gaps in our understanding of how they regulate growth and morphogenetic processes. Further, little is known about how the spatial organization of the pathway is established and maintained and how the Ds-Fat junctions get coordinately remodeled along with other adhesion complexes to allow morphogenesis. To address these critical gaps, we use the *Drosophila* wing disc which provides a robust model system to study this pathway. In the developing Drosophila wing disc epithelium, Ds is expressed in a steep gradient with high expression from the periphery to the very low expression at the center of the wing pouch. In contrast, Fat is mostly expressed uniformly. The interaction between Ds and Fat is modulated by phosphorylation by the Golgi-resident kinase Four-jointed, which is expressed in an opposing decreasing gradient from the center to the periphery. The graded expression of Ds and Fj along with uniform expression of Fat results in an increasing gradient of Fat activity from the center to the periphery and slope of the gradient of Fat activity regulates cell proliferation by influencing Hippo signaling pathway. Membrane localization of Fat and Ds is dynamically regulated, where unliganded Fat is rapidly endocytosed, compared to the liganded population. Given that Ds level is very low at the center of the pouch region, most of the Fat should be unliganded and unstable. Therefore, there must be a mechanism to protect the unliganded Fat from endocytic turnover. We have identified a critical motif in the Fat cytoplasmic domain that bind to a key endocytic adapter and also have identified that the Lix1 homolog Low Fat competitively inhibit Fat endocytosis. Further, we have identified that an intricate interplay between recycling and endocytosis plays a central role in maintaining Fat levels. Taken together, these results indicate that vesicular trafficking provides an important layer of regulation in organization of the Fat signaling pathway. Furthermore, these studies provide novel mechanistic insight into Fat signaling pathway and address several longstanding questions in the field and will help explain the developmental disorders resulting from dysregulation of this pathway.

40 **Bruno1 is required throughout *Drosophila* indirect flight muscle development to regulate cytoskeletal assembly and sarcomere growth** Elena Nikonova[1], Tobias Straub[2], Maria L Spletter[1,31]Physiological Chemistry, Ludwig-Maximilians-Universität München, [2]Ludwig-Maximilians-Universität München, [3]Biological and Biomedical Systems, University of Missouri Kansas City

The differential expression of structural protein isoforms influences cytoskeletal assembly and muscle contractile properties. CELF family RNA binding proteins are important regulators of RNA processing in muscle that are misexpressed in myotonic dystrophy type I. However, it is incompletely understood how CELF proteins influence normal myogenesis, and how misregulation of CELF proteins leads to defects in sarcomere assembly, growth and function. Bruno1 (Bru1, Arrest) encodes a CELF1/2 homolog in *Drosophila* that regulates flight muscle specific alternative splicing. Here we show that Bru1 is required throughout muscle development to regulate cytoskeletal assembly and growth dynamics. During early myofibril formation before 48h APF, using both temporally-restricted RNAi knockdown and overexpression, we show that misexpression of Bru1 leads to disorganization of the actin cytoskeleton, aberrant myofiber compaction and defects in pre-myofibril formation. Developing flight muscles are characterized by gene misexpression and splice isoform switches in diverse structural proteins regulating sarcomere growth and actomyosin interactions, as revealed by transcriptomic and proteomic analyses. Notably, a switch to mature splice isoforms observed during development of control muscle is blocked in flight muscles lacking Bru1. Live-imaging assays confirmed aberrant contractility of *bru1* mutant myofibers already at 48h and 72h APF. By monitoring incorporation of fluorescent actin and myosin proteins during myofibril maturation after 56h APF in *bru1* mutant IFM, we further show that during later stages of myogenesis lateral sarcomere growth is dramatically misregulated, leading to an exacerbation of pre-existing myofibril defects, myofibril fusion and formation of hollow myofibrils. A progression in the severity of cellular and molecular phenotypes from 80h APF to adult distinguishes hypercontraction from earlier growth defects, and temporally restricted rescue can partially alleviate hypercontraction in late pupal and adult stages. Taken together, our data demonstrate that Bru1 regulates cytoskeletal growth and remodeling throughout myogenesis, including cytoskeletal rearrangement necessary for myofibril formation as well as the balance in length versus lateral growth of the sarcomere. Defective RNA processing

due to misexpression of CELF proteins thus causes wide-reaching structural defects and progressive malfunction of affected muscles.

41 **A contractility dependent rigidity transition shapes the curvature of the pupal retina** Jacob Decker[1], Ilaria Rebay[21]Molecular Genetics and Cell Biology, University of Chicago, [2]Ben May, University of Chicago

Many recent studies have demonstrated that epithelial tissues exhibit dynamic changes in their tissue material properties—including viscosity, elasticity, yield stress, and other biophysical moduli—during morphogenesis events. Most work on epithelial morphogenesis has focused on active force generation as a mechanism to create tissue deformations, but the discovery of the importance of tissue material properties in multiple morphogenesis contexts changes this paradigm. Importantly, tissues and organs do not develop in isolation—they develop in crowded spaces and experience varying physical forces from the environment. Tissue material properties are closely related to these external forces as they determine how tissue structures deform in response to an applied force. We are using the pupal retina to study how specific cell behaviors tune tissue material properties, and to assay how external mechanical forces influence developing tissue morphologies. This project specifically focuses on the interplay between cell-scale behaviors and tissue material properties as a mechanism to generate an organ-scale curvature in the retinal epithelium, which eventually confers visual acuity to the mature eye.

Using morphometric analysis of explanted and *in situ* retinal epithelium shapes throughout pupal development, coupled with theoretical analyses adapted from the field of polymer physics, we find that retinal curvature is established via a tissue scale rigidity transition that unfolds over an ~8hr hour window. Cell type specific perturbation of mechanical coupling within the epithelium shows that pigment cells—which make up the edges of the hexagonal ommatidial units—are required for this transition. Mechanical coupling of photoreceptor cells, which occupy the ommatidial core, was found to be dispensable. This increase in rigidity temporally correlates with hexagonal pattern refinement and a build-up of junctional tension within the apical pigment cell lattice. Pigment cell-specific manipulation of actomyosin contractility shows that this increase in junctional tension is a main driver of tissue-scale rigidity. Finally, we are altering the retina's mechanical environment, through a series of genetic manipulations of underlying brain tissue, and will report insights that emerge regarding how regulation of tissue material properties acts in concert with tissue non-autonomous forces to produce functional tissue morphologies.

42 **Novel mechanosensitive junction interactor Gish is required for apical constriction and epithelial folding** Reina Koran, Mo WengUniversity of Nevada Las Vegas

Adherens junctions, the Cadherin-based cell-cell junctions that resist physical tension, often mediate mechanosensitivity during morphogenesis. We showed previously that adherens junctions are strengthened through growing in packing density and size in response to actomyosin contraction during the apical constriction and epithelial folding of Drosophila mesoderm. Such strengthening is essential in connecting actomyosin into a supercellular network as well as protecting tissue integrity. However, the molecular mechanism that mediates this junction strengthening is unknown. We have identified Drosophila casein kinase I gamma, Gilgamesh (Gish), as a potential mechanosensitive junction interactor that strengthens adherens junctions. We found that in the resting state, Gish uniformly localizes to the cell membrane. Upon apical myosin activation, Gish appears to be recruited to junction clusters. Myosin RNAi knockdown and optogenetic inactivation abolishes such cluster recruitment of Gish, while ectopic myosin activation is sufficient to recapitulate the recruitment. Gish cluster localization also depends on adherens junctions, since Gish can no longer be recruited into clusters in mutant embryos that lose core junction components such as alpha-catenin. Importantly, in Gish mutants, adherens junctions in the mesoderm cannot form large clusters in response to apical myosin contraction and appear to be diffuse on the apical surface. Additionally, large quantities of membrane tethers and blebs accumulate on the apical surfaces of these mesoderm cells, resembling loss of junction phenotype. At the tissue levels, apical constriction cannot occur efficiently, leading to failure of epithelial folding. These data suggest Gish is a mechanosensitive junction interactor essential for junction strengthening and epithelial tissue folding.

43 **Ligand-dependent feedback and ligand-independent activation regulate Notch signaling during wing vein patterning in *Drosophila*** Julio Miranda-Alban[1,2], Chyan Rangel[1], Ilaria Rebay[1,21]The Ben May Department for Cancer Research, University of Chicago, [2]Committee on Development, Regeneration and Stem Cell Biology, University of Chicago

The acquisition of cell fates in reproducible spatiotemporal patterns is a fundamental feature of metazoan development. Organ patterning requires meticulously controlled cell-cell interactions, as exemplified in the establishment of the stereotypical vein pattern in the Drosophila wing. While vein specification starts at 3rd instar, the bulk of vein growth,

spatial patterning and boundary refinement occurs during early pupal stages, through ~30h APF. As the early pupal wing elongates proximo-distally, and its dorsal and ventral epithelia appose, EGFR and BMP signaling promote vein formation. These initially rather thick vein regions, which lack clear vein-intervein boundaries, are refined by Delta-Notch signaling between 16-30h APF. Developing vein cells express the ligand Delta, which activates the Notch receptor in flanking cells to repress vein fate, and in turn Delta expression, thus establishing a feedback system that also prevents Notch activation in the veins. While it has been known for decades that reduced Notch signaling results in excessive vein formation and Notch overactivation leads to vein loss, limited understanding of the quality control mechanisms that fine-tune Notch activity in space and time during vein patterning has stymied accurate interpretation of complex vein phenotypes.

In this work we used fluorescent signaling reporters and a transcriptional timer system to examine the spatiotemporal dynamics of Notch signaling and the different modes of Notch activation during wing vein development. We find that recurrent episodes of aberrant signaling are constantly polished by Delta-Notch feedback, well beyond 30h APF. Genetic manipulations of Notch activity show that this feedback mechanism is robust and, while normally actively used only in the vein areas, can be activated anywhere in the tissue under inappropriate/non-physiological Notch signaling levels. Likely contributing to this regulatory scheme, Delta/Serrate-independent low levels of Notch signaling are maintained in the interveins to actively repress vein fate. To our knowledge, this is the first report of a developmental role for ligand-independent Notch activation in non-circulating cells. Further highlighting the sensitivity of vein patterning to Notch activity level and the importance of fine-tuning Notch via quality control mechanisms, our model explains how the degree of *loss of Notch function* predicts whether vein expansion manifests as vein thickening or as ectopic veins.

44 **The physiological consequences of polyploid cells in the *Drosophila* Brain.** Deena Damschroder, Katherine McDonald, Laura ButtittaUniversity of Michigan

As animals age, some cells in the brain undergo a process called endoreplication, which is a variant form of the cell cycle variant mitoses. Endocycles result in cells with nuclei that contain extra copies of the genome, a state termed polyploid. There has been a longstanding correlation between polyploid neurons in the brain and neurodegeneration. Despite this observation, the role polyploid cells have in the brain and the underlying molecular alterations caused by polyploidy are unknown. Polyploid glia and neurons are enriched in the optic lobes of the *Drosophila melanogaster* adult brain and the proportion of polyploid cells increases within the first 3 weeks of adulthood. Polyploid cells are more resistant to cell death after being exposed to exogenous stressors, leading us to wonder whether polyploidy may be an adaptive response that protects against age-accumulated damage and neurodegeneration.

Using the powerful genetics of the *Drosophila* system, we plan to examine the causes and consequences of polyploidy in the *Drosophila* brain. We find that UV-induced DNA damage increases polyploidy, while alterations of lifespan do not, suggesting that the accumulation of polyploid cells during the first three weeks of adulthood may be dependent upon exogenous sources of cellular damage. Our next steps are to manipulate the levels of polyploidy in the brain by altering DNA replication licensing factors, to determine the impacts of polyploidy on animal physiology. The results from our study will help illuminate the roles for polyploid cells in the brain.

45 **Fruit flies and yeast meet at the table: symbiotic mechanisms of interkingdom lipid transfer underlying *Drosophila'*s thermoregulation** Claudia Y Espinoza[1], Emily Behrman[2], Gudrun Ihrke[2], Daniel Milshteyn[3], Jennifer Lippincott-Schwartz[2], Itay Budin[31]Chemistry and Biochemistry, University of California San Diego, [2]Janelia Research Campus, Howard Hughes Medical Institute, [3]University of California San Diego

Organisms rely on efficient thermoregulatory mechanisms to survive fluctuating environmental temperatures. Ectotherms like *Drosophila* lack complex processes to regulate their intrinsic temperature, but must function in low temperature environments. It is still unclear what thermoregulatory strategies *Drosophila* use to adapt to cold environments. Cellular membranes respond to low temperatures by altering lipid composition, for example increasing the number of membrane lipids with polyunsaturated fatty acids (PUFAs) to maintain physiological membrane properties, like fluidity and viscosity. *Drosophila* lacks the desaturase enzymes to produce PUFAs, but several of dietary-yeast species they feed on can synthesize PUFAs. We hypothesize that *Drosophila* forages for PUFA-producing yeast in cold environments for seasonal adaptation. We have found that *D. melanogaster* predominantly associates with PUFAs-producing-yeast species during cold months in a wild population and PUFA composition in the *Drosophila's* body reflects that of the yeast it associates with. In the lab, flies show an olfaction-dependent preference for PUFA-producing yeast in the cold and remain active at low temperatures when fed PUFA-containing yeast. Based off these observations, we have explored mechanisms underlying the symbiotic relationship between *Drosophila* and yeast that determine *Drosophila's*

cold adaptation. Using analysis of yeast strains with altered PUFA metabolism in conjunction with *Drosophila* behavioral assays, we identified isoamyl acetate as a candidate volatile that could allow flies to select for PUFA-producing yeast. Our goal is to understand how *Drosophila* integrates such signals and compounds to adapt to cold temperatures. In parallel, we are exploring how PUFA-rich diets promote cold activity and survival. Lipidomic analysis of flies fed with different yeast diets identified a class of ether phospholipids - plasmalogens – as the destination of dietary PUFAs in the *Drosophila* body. We are currently investigating how the synthesis and biophysical properties of plasmalogens regulate *Drosophila* cold activity.

46 **Enhanced longevity through muscle hypercontraction** Saki Naito, SaKan YooRIKEN

How lifespan of organisms is regulated is a fundamental and unresolved question. To reveal mechanisms that regulate lifespan, we performed an EMS-based genetic screening. Among approximately 900 EMS mutants, we identified a gain of function mutant of *Myosin heavy chain* (*Mhc*) as a longevity mutant that has enhanced lifespan. This mutant exhibits disrupted myofibrils and locomotion defects due to muscle hypercontraction at the young age. In spite of this apparently unhealthy feature, the *Mhc* mutant delays manifestation of the aging phenotypes such as protein aggregation and sensitization to stresses. Transcriptome analyses demonstrate that the *Mhc* mutant highly upregulates *Activity-regulated cytoskeleton-associated protein 1* (*Arc1*), which is a secreted factor. Inhibition of Arc1 suppresses the *Mhc* mutation-mediated lifespan extension. We propose that the *Mhc* mutation-mediated muscle hypercontraction generates hormesis effects to the organism through Arc1, which leads to longevity. This work provides new insight into how organismal lifespan is regulated.

47 **Reduced expression of the modifier *CG4168* alleviates metabolic phenotypes caused by *Sirt1* loss of function** Rebecca PaluBiological Sciences, Purdue University-Fort Wayne

Insulin resistance is a dangerous complication that can affect both Type I and Type II diabetics and is linked to inflammation, metabolic imbalance, and tissue damage. Improved knowledge of the genetic risk factors associated with insulin resistance will enable physicians to better identify and treat at-risk patients. One well-known genetic risk factor is *Sirt1*. This protein deacetylase regulates metabolic pathways in response to the energetic state of the cell. Prior work shows that loss of *Sirt1* expression in the fly is associated with progressive obesity, diabetes, and insulin resistance. This is recapitulated in mammalian models and in human patients. However, in all models and patients, the impact of *Sirt1* varies depending on genetic background. To identify genetic risk factors of specifically hyperglycemia in a *Sirt1* loss-of-function model, we performed a natural genetic variation screen. We identified more than 100 conserved candidate genes, several of which have roles in the secretion, maintenance, and regulation of insulin signaling. One of these is the uncharacterized gene *CG4168*. This gene encodes a putative insulin-like growth factor binding protein, and reducing its expression was shown to suppress hyperglycemia induced upon *Sirt1* loss-of-function. We hypothesized that the CG4168 protein normally stabilizes insulin-like peptides (ilp) in circulation, leading to chronic hyperinsulinemia in the *Sirt1* loss-of-function model and ultimately contributing insulin resistance. Reducing expression of *CG4168* would therefore reduce ilp stability in circulation, slowing or stopping the development of insulin resistance. We observed that reducing expression of *CG4168* in the fat body of *Sirt1* loss-of-function flies reduced hyperglycemia and obesity as compared to the *Sirt1* loss-of-function alone. We intend to determine if this effect is conserved in the insulin producing cells, as well as whether circulating ilp levels are altered in the absence of *CG4168*. If so, it is possible that the orthologues of *CG4168*, like the human *IGFALS*, may be good candidates as therapeutic targets in insulin resistance.

48 **Drosophila embryos allocate lipid droplets to specific lineages to ensure punctual development and prevent oxidative stress** Marcus Kilwein[1], T Kim Dao[2], Michael A Welte[21]University of Rochester, [2]Biology, University of Rochester

Mothers endow their embryos with a diverse set of nutrient reserves. These nutrients are transient stores including lipid droplets (LDs), glycogen granules and yolk-protein vesicles which provide the embryos with energy that fuels development and carbon scaffolds for anabolism. LDs are the only nutrient storage structure deposited into the blastoderm; the others are inherited by the yolk cell. Proper LD allocation requires a multistep process that employs actin-driven cytoplasmic streaming and active transport along microtubules.

To investigate why LDs occupy this privileged position, we employed mutations that lead to LD deposition into the yolk cell. We show that *Jabba* null embryos have an inappropriate association between LDs and glycogen granules that leads to LDs being dragged with glycogen into the yolk cell. Previous work had shown that *klar* null embryos also deposit LDs in the yolk cell, but via an entirely different mechanism, impaired microtubule-based motion.

We found that in *Jabba* and *klar* null embryos both triglyceride consumption and the duration of embryogenesis are disturbed. The yolk cell localized LDs persisted through hatching, demonstrating that correct tissue inheritance is crucial to their consumption, and embryogenesis was delayed. To test whether this delay results from reduced LD consumption or excess LDs damaging the yolk cell, we examined embryos from *dPLIN2* null mothers; these embryos receive a reduced maternal supply of LDs and exhibit significant developmental delays, strongly suggesting that the delay in *Jabba* and *klar* mutants is due to reduced LD accessibility.

To elucidate how embryos cope with this LD-deprivation, we performed RNA sequencing and proteomics and found significant alterations in the metabolic proteome. All three mutants display higher levels of both peroxidated lipids and glutathione as well as upregulation of genes which alleviate oxidative stress. These observations indicate that LD-deprivation results in oxidative stress and that the embryos mount an active response to combat it. Indeed, zygotic RNAi for Glutathione Synthase and Glutathione-S transferase T4 reduced hatching success in a *Jabba* mutant background but not in the wild type. Our work demonstrates that embryos allocate LDs into specific lineages to ensure optimal development and prevent oxidative damage.

50 **Hypoxia-dependent regulation of epithelial tissue growth.** Abhishek SharmaBiochemistry and Molecular Biology, University of Calgary

Our cells and organs need oxygen from the air we breathe in order to survive and function. However, in certain disorders - such as stroke, heart disease and cancer - tissues are often deprived of oxygen. *Drosophila* larvae provide an excellent in vivo model to study adaptive responses to hypoxia as they have evolved to live in naturally low oxygen conditions. We previously identified two mechanisms that promote organismal hypoxia tolerance – activation of the FOXO transcription factor and Tsc1/2-mediated suppression of TOR kinase signaling. Here we examine how both mechanisms operate to control organ-level growth and proliferative adaptation to hypoxia. To do this we examined *tsc1* and *foxo* function in the developing larval imaginal disc epithelial tissues. Cell clones mutant for both *tsc1* and *foxo* show a tumor-like overgrowth phenotype under normal growth conditions, and we found that this overgrowth is exacerbated under hypoxia and leads to altered tissue patterning. We also found that larvae bearing *tsc1/foxo* tumors in their eye imaginal discs had reduced survival in hypoxia. We saw that *tsc1/foxo* double mutant tumor clones are susceptible to high amount of apoptosis as compared to their wildtype counterparts and that this cell death is also further exacerbated under hypoxic conditions. We also observed upregulation of autophagy in tsc1/foxo mutant clones using Lysotracker staining (used to stain acidic vesicles such as lysosomes and late autophagosomes) specifically in hypoxia. These results suggest that *tsc1/foxo* clones growing in hypoxia experience high levels of proteotoxic stress and, as a result, increased cell death. Interestingly, overexpression of the cell death inhibitor P35 in both *tsc1/foxo* clones and neighbouring wildtype clones supressed the overgrowth of *tsc1/foxo* clones in hypoxia, suggesting that the induction of cell death is required for tumor overgrowth in hypoxia. We are currently exploring the mechanisms by which cell death is promoting this growth. The behaviour of *tsc1/foxo* double mutant clones under hypoxia recapitulate various tumor phenotypes such as high proliferation and apoptosis. Hence, our work will help uncover how hypoxia impacts tumor growth.

51 **Depleting CRL4 E3 ligase Mahjong/DCAF1 induces *Minute*-like cell competition through transcription factor Xrp1, independently of cell polarity genes** Amit kumar, Nicholas E. BakerGenetics, Alber Einstein College of Medicine

Cell competition entails the removal of viable cells when present next to fitter cells. Mahjong (Mahj), a substrate receptor for ubiquitin E3 ligation, binds to lethal giant larvae (*lgl*), a neoplastic tumor suppressor, and regulator of the apicobasal polarity of the cell. Loss of either *mahj* or *lgl* in a mosaic tissue induces cell competition when present next to wild-type cells. We show that the bZip-domain transcription factor Xrp1, which eliminates *Minute* cells (heterozygous for Ribosomal protein gene mutations), is required for the competition of *mahj* mutant cells, but is dispensable for *lgl*-mediated competition. Xrp1 expression in *mahj* mutant cells results in lower translation, slower growth, and activation of cell competition-associated signaling pathways. Apart from *mahj*, the knockdown of other CRL4 E ligase subunits (DDB1 and Cul4) or proteasome subunits also induces *Minute*-like cellular phenotypes. Thus, our findings dissociate *mahj*-mediated cell competition from apical-basal polarity and link it to E3 ligase function. We will discuss whether cell competition of *mahj* mutant cells serves as protection against genomic instability.

52 **Ionizing Radiation induces cells with past caspase activity that contribute to the adult organ in *Drosophila* and show reduced Loss of Heterozygosity (LOH)** Sarah I Colon Plaza[1], Tin Tin Su[21]University of Colorado Boulder, [2]Molecular, Cellular and Developmental Biology, University of Colorado, Boulder

There is increasing recognition that cells may activate apoptotic caspases but not die, instead displaying various

physiologically relevant consequences. We know very little, however, of mechanisms that underlie the life/death decision in a cell that has activated apoptotic caspases. By optimizing a published reporter of past caspase activity, we have been able to visualize such cells that result specifically from exposure to ionizing radiation (IR) 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 wing imaginal discs of Drosophila larvae and that the wing disc stage of development impacts the number of XPAC. Our data shows that in early stages of development (no wing folds) there are more XPAC cells. Furthermore, this reporter has allowed us to gather pre-liminary data on genes that might influence the number of XPAC. Axin, a negative regulator of wingless signaling, and mastermind deletion (mamΔ), to negative regulate Notch signaling pathway, decrease the number of XPAC cells in early developmental stages but have no impact in late development stages. These data suggest that wingless and Notch signaling regulate whether cells with caspase activity live or die depending on the stage of development. We also found that heterozygotes of H99 chromosomal deficiency that removes pro-apoptotic genes hid, rpr and grim show reduced number of XPAC cells. Yet, XPAC cells appear in stereotypical patterns that do not follow the pattern of IR-induced apoptosis, suggesting additional controls at play. By following irradiated larvae into adulthood, we found that XPAC cells contribute to the adult wing. By combining a reporter for past caspase activity with mwh, an adult marker for Loss of Heterozygosity (LOH), we addressed the relationship between XPAC and genome stability after irradiation. We find that XPAC cells show reduced LOH relative to the rest of the wing, suggesting a physiological role for non-lethal caspase activity during recovery from radiation damage.

53 **Sex-specific differences in the activation of the Integrated Stress Response** Melissa Mychalczuk, Lydia Grmai, Deepika VasudevanCell Biology, University of Pittsburgh

When an organism is subjected to stressors such as nutrient deprivation, it adapts in order to survive via the activation of stress response pathways. The Integrated Stress Response (ISR) is an evolutionarily conserved signaling pathway that alleviates stress by regulating mRNA translation and selectively promoting expression of stress-alleviating genes via the transcription factor ATF4. Highly metabolic tissues, such as fat, rely upon constitutive activation of ATF4 for their homeostasis. Metabolic differences between males and females have been well documented across many species. However, whether there are sex-specific differences in stress tolerance, and if this is mediated by ATF4, are unknown. In this work, we use the Drosophila melanogaster larval fat body, which is analogous to mammalian liver and adipose tissue, to study sex differences in ISR activation and stress tolerance. We found that a reporter for ATF4 activity, 4E-BPintron-DsRed, is substantially higher in fat bodies from wandering third instar female larva in comparison to male larva. Further qPCR analysis of ATF4 mRNA and ATF4 targets (Thor) confirmed that ISR activation was higher in the female fat body than the male fat body. To test if these differences in basal ISR activation influence organismal tolerance to stress, we induced nutrient deprivation stress by driving expression of a bacterial methionine-degrading enzyme, methioninase, specifically in the fat body. Consistent with previously published data, we found that methionine deprivation extended the lifespan of both female and male adults. Interestingly, in these lifespan assays we also observed that female methionine-deprived adults lived longer than their male counterparts. Our preliminary results also show greater loss of lipid content in males than in females in response to fat body-specific methionine deprivation. Based on these observations, we hypothesize that female flies experience higher levels of ISR activation and consequently have a higher tolerance to stress induced by nutrient deprivation than male flies. We are currently examining if canonical sex determinants such as doublesex orchestrate sex differences in ISR activation. In summary, this work provides a basis for understanding the molecular mechanisms that underlie sex differences in stress tolerance.

54 **Xrp1/Irbp18 heterodimer governs the stress response program to spliceosome dysfunction** Dimitrije Stankovic, Luke Tain, Mirka UhlirovaInstitute for Genetics/CECAD Cologne, University of Cologne

Pre-mRNA splicing catalyzed by the spliceosome represents a vital step in regulating gene expression and maintaining genome integrity. In contrast, mutations in spliceosome components or their dysregulation have been associated with various human diseases, termed spliceosomopathies. Here we show that imaginal cells experiencing scarcity or mutations in U5 snRNP spliceosome components suffer from extensive transcriptional remodeling and accumulation of highly mutagenic R-loops resulting in activation JNK and p53 signaling and cell cycle arrest. Despite genomic instability and robust stress response, U5 snRNP deficient cells increased protein translation and cell size, causing intra-organ growth disbalance before being gradually eliminated via apoptosis. We identify the Xrp1/Irbp18 heterodimer as the primary driver of transcriptional and cellular stress program downstream of U5 snRNP malfunction. Knockdown of *Xrp1* or *Irbp18* in U5 snRNP compromised cells attenuated JNK and p53 activity, restored normal cell cycle progression and growth, and inhibited cell death. Reducing Xrp1/Irbp18, however, was insufficient to rescue the splicing defects and the

organismal lethality, highlighting the importance of proper splicing in maintaining cellular and tissue homeostasis. In summary, our work provides novel insights into the crosstalk between pre-mRNA splicing and the DNA damage response and defines the Xrp1/IRBP18 heterodimer as a key sensor of spliceosome malfunction.

55 **Increased intracellular pH promotes cell death in the developing *Drosophila* eye** Rachel Ann D Soriano[1], Juan M Reyna Pacheco[2], Joanne Mendez[2], Bree Grillo-Hill[11]Biological Sciences, San Jose State University, [2]San Jose State University

Regulated cell death is essential during development to precisely pattern tissues and avoid developmental errors. Dysregulation of cell death is associated with pathologies including cancer (reduced cell death) and neurodegeneration (increased cell death). Dysregulated intracellular pH (pHi) dynamics are also associated with these diseases, where cancer cells have constitutively higher pHi than normal cells while degenerating neurons have lower pHi. Supporting this, *in vitro* experiments show that apoptotic cell death caspase enzymes have increased activity at low pH. Together, these observations led to the current view that cell death is enhanced at low pHi and inhibited at higher pHi. Our objective is to directly test this prediction *in vivo*. We used the *GMR-GAL4* driver to overexpress the Na-H exchanger *DNhe2* in the eye (*GMR>DNhe2*), which increases pHi and results in a smaller, mispatterned adult eye. We also used another transgenic line to overexpress the proto-oncogene *Myc* (*GMR>Myc*), which regulates cellular processes such as proliferation and cell growth. Using confocal microscopy, we imaged pupal eyes, performed cell counts, and analyzed our results for statistical significance. We found a significant decrease in cell number, from an average of 15 cells in control to 12.4 cells with *GMR>DNhe2*. We previously published increased proliferation in *GMR>DNhe2*, suggesting increased cell death reduces cell number. We next tested for genetic interactions between *GMR>DNhe2* and cell death genes. We found that pH-dependent cell death is caspase-independent, but p53-dependent and requires autophagy genes. Together, these data are inconsistent with apoptosis but suggest that the cells are eliminated through autophagic cell death. We also identified a genetic interaction where co-expression of *Myc* suppresses the *DNhe2*-induced rough eye and restores cell number to 15, similar to control. We are also testing molecular markers, and preliminary data suggest marker expression consistent with increased autophagy in *GMR>DNhe2* eyes. Together, our findings will elucidate mechanisms for pH regulation of conserved, critical developmental processes and provide evidence for new paradigms in growth control.

56 **A pseudokinase-mediated feedback loop regulates neuronal stress responses and links proteostasis defects to sleep behavior** Shashank Shekhar, Andrew T Moehlman, Michael Ewnetu, Charles Tracy, Helmut KramerUT Southwestern Medical Center

Retrograde signals are key for organizing the activity of neuronal circuits. We have discovered a secreted pseudokinase that regulates in a cell non-autonomous manner stress responses in neurons and affects sleep quality. To investigate the molecular mechanisms of neuronal stress responses, we use a reversible in-vivo model. In the Drosophila visual system, extended ambient light exposure (LL) induces structural plasticity of rhabdomeres, the sensory organelles of photoreceptors. These responses are reversible and therefore offer an excellent opportunity to study signaling events controlling neuronal homeostatic responses (1,2). Here, we identify *allnighter* (*aln*) as a novel gene required for the regulation of neuronal homeostasis. *aln* encodes a secreted pseudokinase that is functionally conserved in humans. Flies mutant for *aln* have normal visual responses when maintained under a regular 12h:12h light:dark cycle (LD), but under LL lose rhabdomere structural integrity and postsynaptic responses. This misadaption is transient; photoreceptors recover when returned to LD. *aln* phenotypes are rescued by expression of Aln or its closest human homolog, the divergent protein kinase Dipk1C, independent of its kinase activity. The *aln* gene is expressed in many neurons but, surprisingly, not in photoreceptors. Instead, it is secreted by lamina neurons, most prominently L4, and retrogradely regulates homeostatic responses in photoreceptors including UPR and autophagy, two key stress pathways. Surprisingly, Tetanus Toxin light chain or Shi^{ts1}-driven suppression of synaptic activity specifically in photoreceptors drastically decreased Aln expression in L4 lamina neurons, and other brain regions as well. Aln function is not restricted to the visual system, as *aln* brains display widespread expression of stress markers, including Ref(2)p and ATF4, and show disrupted sleep behavior. Together, our data suggest that the Aln pseudokinase contributes to a feedback loop that is responsive to visual activity and regulates neuronal stress responses.

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57 **Differential neuronal vulnerability associated with C9orf72 expansion toxicity** Teresa Niccoli, Dunxin Shen,

Carlo FrigerioUCL

Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are both devastating neurodegenerative diseases having no cures. A hexanucleotide repeat mutation in the C9orf72 gene is the most common genetic cause of those two diseases. Like many other neurodegenerative diseases, ALS and FTD display selective neuronal vulnerability: only some neuronal populations succumb to disease, even though the toxic species are ubiquitously expressed.

However, why and how different types of neurons react differently in the disease progression remain unclear. In order to identify which neuronal populations are selectively depleted in response to the disease onset and analyse which pathways are activated in vulnerable and resistant neuronal populations respectively, we carried out single-cell RNA sequencing on the brains of a fly model of expressing 36 hexanucleotide repeats.

We have analysed the transcription profile of single brain cells following induction over the course of the developement of toxicity, to allow us to understand how neuronal responses to the repeats develops as disease progresses. We have identified pathways that are uniquely activated in vulnerable neurons and pathways associated with resistant neurons. This work goes some way to explaning why only some neurons succumb to disease in patients and might allow the delovpment of more targetted therapies.

58 **Dual roles of an ABCA transporter in phagocytosis-dependent neurodegeneration in *Drosophila melanogaster*** Xinchen Chen, Ankita Sarkar, Hui Ji, Bei Wang, Chun HanMolecular Biology and Genetics, Cornell University

Lipid homeostasis is essential to the function and survival of neurons. Consequently, dysregulation of lipid homeostasis in the nervous system is a frequent cause of neurodegeneration. Lipid transporters are major regulators of lipid homeostasis by moving lipids inside and between cells. Although certain members of the ATP-binding cassette A (ABCA) protein subfamily are associated with neurodegenerative diseases, how these lipid transporters are involved in neurodegeneration by regulating specific aspects of neuronal lipid homeostasis remains poorly understood. By screening ABCA homologues in Drosophila, we found that *Engulfment ABC Transporter in the ovary* (*Eato*) plays dual roles in phagocytosis-dependent degeneration of larval somatosensory neurons: In neurons, *Eato* loss-of-function (LOF) results in severe dendrite and axon degeneration, which is rescued by blocking engulfment activities of nearby phagocytes and by suppressing the externalization of the “eat-me” signal phosphatidylserine (PS) on the neuronal membrane; in phagocytes, *Eato* loss-of-function (LOF) makes phagocytes less capable of sensing PS-exposing neurons and impairs PS-mediated neurodegeneration. Moreover, Eato is localized on the plasma membrane and functions epistatically upstream of the engulfment receptor Draper in phagocytes. Surprisingly, we found that the defects caused by *Eato* loss in both neurons and phagocytes are at least partially due to aberrant PS exposure and that the level of PS exposure on phagocytes is negatively correlated with phagocytic activity. Thus, Eato plays distinct roles in neurons and phagocytes by suppressing and promoting neurodegeneration, respectively, both via preventing PS externalization on the surface of the cell. These results reveal novel mechanisms by which ABCA proteins regulate lipid homeostasis and neuron-phagocyte interactions during neurodegeneration.

59 **Loss of Syndecan disrupts peripheral glia** Duo Cheng, Vanessa AuldZoology, University of British Columbia

Glia are irreplaceable components for the nervous system development and function. In the *Drosophila* larva, three distinct glial types surround each peripheral nerve, providing support, protection, and insulation. However, the cellular mechanisms each glial layer utilizes to communicate with each other and the extracellular environment isn’t well characterized *in-vivo*. Here, we investigated the role of a heparan-sulfate proteoglycan, Syndecan (Sdc), in regulating glial cell function and development in the *Drosophila* peripheral nervous system. Using super-resolution microscopy, we found Sdc is distributed throughout the PNS glial layers with a concentration in the perineurial glia that cover both the PNS and the CNS (including the ventral nerve cord (VNC) and brain lobes). When Sdc was knockdown in all glia, we observed a significant elongation in the VNC, a phenotype similar to loss of integrin focal adhesions, which suggests Sdc plays a key role in regulating glial-ECM adhesion. We also observed several distinct phenotypes within the peripheral glia with loss of Sdc. To better understand Sdc’s function in the peripheral nerve, we specifically knocked down Sdc in each glial layer. Loss of Sdc in wrapping glia resulted in the absence of axon ensheathment and loss of Sdc in the subperineurial glia led to localized cell hypertrophy and disruption of septate junction protein distribution. This data suggests that Sdc plays a role in maintaining the internal glial layers of the peripheral nerve and may play a role in glial

sheath stability and blood nerve-barrier integrity. Finally we analyzed the effects of knockdown of Sdc in the perineurial glia (PG), the outer glial layer in direct contact with the overlying ECM. Loss of Sdc resulted in incomplete ensheathment and migration defects in the PG of the abdominal nerves. These phenotypes mirror those we previously observed with the loss of integrins in the PG. To test the potential synergistic interaction between integrin and Sdc, we used a mutation in the integrin β-subunit (*myospheroid*) in combination with Sdc RNAi in the PG. Reduction of both proteins enhanced the VNC elongation phenotype and the loss of ensheathment of the peripheral nerves. However, the reduction of Sdc and integrin together did not enhance the perineurial glial migration defect suggests that Sdc affects perineurial migration using a mechanism independent of integrins. Overall we have identified Sdc as a key component in proper ensheathment and function of multiple glial cell types in the peripheral nerve.

60 **Developmental emergence of sleep rhythms enables long-term memory capabilities in *Drosophila*** Amy Poe[1], Lucy Zhu[1], Patrick McClanahan[2], Milan Szuperak[1], Ron Anafi[1], Andreas Thum[3], Daniel Cavanaugh[4], Matthew Kayser[11]University of Pennsylvania, [2]KU Leuven, [3]Leipzig University, [4]Loyola University Chicago

A major unanswered question in chronobiology is how maturation of the circadian clock is tied to the developmental emergence of behavioral and physiological rhythms. While molecular circadian rhythms are evident during early development, consolidated sleep-wake patterns do not emerge until far later. Normal maturation of sleep and circadian rhythms during early life is important for brain development; disruptions to sleep and rhythms during development are a common co-morbidity in neurodevelopmental disorders. Using the LarvaLodge platform for monitoring sleep in developing *Drosophila* larvae, we have previously shown that sleep in 2nd instar larvae (L2) is not under circadian control. Here, we identify the precise developmental timepoint when the circadian clock begins to regulate sleep in *Drosophila*, leading to the emergence of sleep rhythms at the early 3rd instar stage (L3). A neural activation screen of peptidergic cells reveals that manipulation of activity in *Diuretic hormone 44* (Dh44) neurons in the brain bidirectionally modulates sleep-wake at L2. During this period, Dh44 cells do not exhibit rhythmic activity patterns or receive input from the clock network. However, at the early L3 stage, we find that Dh44 activity begins to cycle and Dh44 neurons form anatomical and functional connections with the DN1a clock neurons. *CCHamide-1* (CCHa1) signaling between DN1as and Dh44 neurons is necessary for the emergence of sleep rhythms. Finally, sleep is a critical regulator of memory formation in adulthood, but it is unknown when and how these processes become coupled. We demonstrate that while both L2 and L3 larvae exhibit short-term memories, genetic manipulations that disrupt sleep rhythms selectively block long-term memory capacity at L3. Together, our data indicate that development of a circuit bridge between DN1a clock cells and Dh44 arousal neurons drives the emergence of sleep rhythms, facilitating enhanced cognitive capabilities.

61 **Homeodomain proteins hierarchically specify neuronal diversity and synaptic connectivity** Chundi Xu[1], Tyler Ramos[1], Edward Rogers[2], Michael Reiser[2], Chris Doe[11]University of Oregon, [2]HHMI Janelia Research Campus

Our brain comprises a vast diversity of neuron types with each embedded in neural circuits which enable us to perceive and respond to the world. Transcription factors regulate both neuronal fate and circuit formation, yet it remains unclear how these two processes are coordinated. *Drosophila* lamina neurons are an ideal system for tackling this question. There are five lamina neurons (L1-L5). These include the «core» L1-L3 neurons that generate the evolutionarily conserved behavioral response to light increments/decrements, and the poorly understood L4 and L5 neurons. L4 and L5 neurons express a combination of homeodomain TFs: Brain-specific homeobox (Bsh) and Apterous for L4; Bsh and Pdm3 for L5. Here we show that Bsh is initiated in lamina precursors where it specifies L4/L5 fate and prevents expression of the homeodomain TF Zfh1 to suppress L1/L3 fate. Furthermore, Bsh cooperates with Notch signaling which is activated in newborn L4 but not L5 to specify L4 and L5 neuronal fate distinctively. Subsequently, Bsh acts in L4 neurons to activate Apterous and initiate a feed-forward loop to express the synapse recognition molecule DIP-β, in part by Bsh direct binding a DIP-β intron. Thus, homeodomain TFs function hierarchically: primary homeodomain TF (Bsh) first cooperates with Notch signaling to specify distinct neuronal fate, and subsequently acts with secondary homeodomain TF (Ap) to activate DIP-β, thereby generating precise synaptic connectivity. Our data provide a novel model by which homeodomain TF networks specify neuronal fate and synaptic connectivity. Our work also has evolutionary implications. Bsh is required to generate lamina neuronal diversity (adding L4/L5 neurons to the core L1-L3 neurons), suggesting a novel and testable hypothesis that neural circuits evolve more complexity by adding the expression of a primary homeodomain TFs such as Bsh.

62 ***Drosophila* Dprs and DIPs are GPI-anchored and this modification contributes to their subcellular localization** Meike Lobb-Rabe*, Viola I Nawrocka*, Engin Özkan, Robert A CarrilloUniversity of Chicago

The *Drosophila* Dpr and DIP proteins belong to the immunoglobulin superfamily of cell surface proteins (CSPs), and hetero- and homophilic interactions have been implicated in a variety of neuronal functions including synaptic connectivity, cell survival, and axon fasciculation. However, the signaling pathways underlying these diverse functions are unknown. To gain insight into Dpr-DIP signaling, we sought to examine how these CSPs are associated with the membrane. Specifically, we asked whether Dprs and DIPs are integral membrane proteins, or instead, if they are membrane anchored through post-translational modifications. Here, we expressed all Dprs and DIPs in S2 cells and used Phospholipase C-mediated cleavage to examine glycosylphosphatidylinostitol (GPI) anchoring. We demonstrate that Dprs and DIPs are GPI-anchored to the membrane and validate some family members in vivo using Drosophila larvae. Additionally, GPI cleavage abrogated aggregation of S2 cells expressing cognate Dpr-DIP partners. To test if the GPI anchor affects Dpr-DIP localization, we replaced it with a transmembrane domain which perturbed sub-cellular localization in motor neurons. Overall, these data suggest that membrane anchoring of Dprs and DIPs through GPI-linkage is required for localization and that Dpr-DIP signaling likely will require transmembrane co-receptors.

63 **The viral like transfer of Copia, a Drosophila retrotransposon, across the synapse antagonize dArc1 at the larval neuromuscular junction** Peter G M'Angale[1], Adrienne Lemieux[2], Alfred Simkin[1], Cong Xiao[1], Shuhao Wang[1], Jasmine Graslie[1], Angela Jimenez[1], Vivian Budnik[1], Travis Thomson[11]Neurobiology, UMass Chan Medical School, [2]Neurobiology, University of Massachusetts Chan Medical School

Transposable elements make up close to 50% of the genome commonly known as "junk DNA" with majorly unknown functions. In previous work from our lab, it was shown that the *activity regulated cytoskeleton-associated protein 1 (dArc1)* together with *Copia*, were enriched in Drosophila S2 cells-derived extracellular vesicles. It was shown that dArc1 was a domesticated retrotransposon fragment that exhibited viral-like trafficking of its mRNA across the synaptic bouton to regulate structural synaptic plasticity at the larval neuromuscular junction (NMJ). Enriched in tandem with dArc1 was Copia, a bona fide retrotransposable element that we show employs a similar transfer of its mRNA like dArc1 and similarly utilizes extracellular vesicles to traffic across the synapses. The pre-synaptic knockdown of dArc1 leads to less structural synaptic plasticity while that of Copia^{gag} results in abnormal NMJ development, increased plasticity, and a marked activity derived phenotype. EM studies confirm that Copia^{gag} forms capsids and is in S2 derived EVs, while through digital PCR and immunofluorescence microscopy we determined that there is an enrichment of copia^{gag} mRNA and protein in the larval brains when compared to other somatic tissue. We observe that copia^{gag} acts antagonistically to dArc1 and together regulate structural synaptic plasticity at the larval NMJ. This, we believe is the first report to document a physiological role for a retrotransposon at synapses.

64 **Mapping transcriptomes to connectomes uncover matching synaptic determinants** Yerbol Z. Kurmangaliyev[1,2], Juyoun Yoo[2], Mark Dombrovski[2], Parmis Mirshahidi[2], Aljoscha Nern[3], Samuel A LoCascio[2], S. Lawrence Zipursky[21]Brandeis University, [2]HHMI, University of California Los Angeles, [3]HHMI, Janelia Research Campus

How do neurons discriminate their synaptic partners from a myriad of other neurons that they encounter during brain development? In genetically hard-wired circuits, this recognition is thought to be controlled by complex arrays of cell adhesion molecules on the surfaces of developing neurons. However, the identification of matching receptor-ligand pairs between synaptic partners that control synaptic specificity has been notoriously challenging. Here, we integrated the detailed synaptic connectome, single-cell transcriptomes, and protein interactomes of cell adhesion molecules in the Drosophila visual system to identify receptor-ligand pairs contributing to synaptic specificity. Currently, our coupled transcriptome-connectome map of the visual system includes ~100 morphologically defined cell types that were matched to transcriptional clusters. This map provides the near-complete set of expressed genes for both sides of many dozens of synaptic connections in the circuit.

We focused on a circuit in which genetically closely related neurons make well-defined binary wiring choices. We found that two homologous receptor-ligand pairs of Beat/Side families of proteins match synaptic partners and define the architecture of two parallel pathways in the motion detection circuit. The genetic analysis shows that interactions between presynaptic Side-II and postsynaptic Beat-VI regulate synaptic specificity in this circuit. Our findings demonstrate that sharp differences in a subset of matching receptor-ligand pairs are sufficient for the differential recognition of synaptic targets by closely related neurons. As each neuronal cell type expresses a unique combination of 14 Beat and 8 Side proteins during circuit assembly, we propose that other receptor-ligand pairs of these families also contribute to synaptic specificity throughout the Drosophila brain. This work expands the diverse repertoire of immunoglobulin-superfamily of proteins that have been shown to regulate synaptic specificity in the Drosophila brain (e.g., Dscam, DIP/Dpr, and now Beat/Side families). Our study provides a framework for combining molecular and

connectivity maps of neural circuits to uncover the molecular strategies underlying synaptic specificity.

65 **Spontaneous network activity during motor circuit development** Arnaldo Carreira-Rosario[1,2], Chris Q Doe[3], Thomas Clandinin[11]Neurobiology, Stanford University, [2]Biology, Duke University, [3]University of Oregon

All nervous systems become spontaneously active during early development, before interacting with the world. This patterned spontaneous network activity (PaSNA) is necessary for the formation of neural circuits. How SNA initiates, and how this initial activity influences subsequent circuit formation, remain unclear in any system. To study this evolutionarily conserved process, we are using the *Drosophila* embryo as a model. In this powerful genetic system, early stages of neurodevelopment are well characterized, motor behaviors can be quantified, and an electron microscope-generated connectome of the entire central nervous system exists, providing a unique opportunity to study PaSNA and its impact on mature behavior. Previously, we combined genetics with calcium imaging to uncover that proprioception, a universal sensory input, acts as a "brake" on PaSNA during development. This finding showed that sensory input plays a critical role at the earliest stages of circuit formation. Using optogenetics, we transiently perturbed early neural activity to demonstrate that PaSNA shapes foraging behavior. This represents one of the first examples that directly links early neural activity to motor behavior. More recently, we discovered that neuropeptidergic signaling, a mode of broadcasting neural information, plays a key role in modulating PaSNA. We identified a specific subset of peptidergic neurons that become intrinsically active before and during PaSNA. Blocking the production of the specific neuropeptide in these neurons weakens SNA, demonstrating that this neuropeptide acts as an "accelerator" of PaSNA during development. We are currently investigating how a specific population of peptidergic neurons becomes intrinsically active and the downstream circuit mechanism for how this signal accelerates PaSNA. Our work is beginning to uncover the cellular and molecular "knobs" that tune the neural activity of the developing fly brain and shape mature behavior.

66 **Tdrd5l promotes male identity in germline stem cells** Caitlin Pozmanter, Mark Van DorenJohns Hopkins University

Tudor-domain containing proteins are conserved across the animal kingdom for their necessary functions in germline development including post-transcriptional gene regulation. Previously our lab identified Tudor domain containing protein5-like (Tdrd5l), which localizes to a perinuclear body that is adjacent to, but distinct from, the Vasa-positive nuage. *Tdrd5l* promotes male germline identity in the *Drosophila* testis but is repressed by the RNA binding protein Sex lethal (SXL) in the undifferentiated female germline. Interesting, Tdrd5l is also expressed in the differentiating germline in both sexes, indicating that it may also act to control gametogenesis in both sexes. Currently, we are working to identify additional protein and, possibly, RNA components of the cytoplasmic body that Tdrd5l defines using proximity biotinylation.

We are also working to understand how Tdrd5l regulates male germline identity. Immunostaining for Tdrd5l shows expression in the larval germ cells and adult GSCs, and *Tdrd5l* interacts with germline sex determination genes in a manner consistent with promoting male identity. *Tdrd5l* mutant adult testes exhibit dramatic germline loss which is often seen in mutants for sex determination factors. Due to this germline loss we used 3rd instar larval males in addition to newly enclosed adults to investigate sex-specific GSC characteristics in *Tdrd5l* mutants. One critical difference between male and female GSCs is their response to the Jak/Stat pathway, where male GSCs respond to this pathway while female GSCs cannot. In *Tdrd5l* mutant male GSCs we observed a reduction of Stat immunostaining, indicating a loss of Jak/Stat response more typical of female GSCs. Male GSCs also exhibit localization of one centrosome to the hub-GSC interface, whereas in female GSCs have the spectrosome localized in this position. In *Tdrd5l* mutants we see loss of centrosome localization, and increased spectrosome localization, to the hub-GSC interface. Together, these results indicate that *Tdrd5l* mutant male GSCs lose male identity and exhibit characteristics of female GSCs. Currently, we are conducting experiments to determine how Tdrd5l functions to regulate male GSC identity and whether Tdrd5l is sufficient to masculinize female GSCs.

67 **A pioneer factor regulates somatic sex reversal in the adult *Drosophila* testis** SNEH HARSH[1], Christine Rushlow[2], Erika Bach[31]New York University, [2]NYU, [3]NYU School of Medicine

Sexual identity of stem cells must be maintained throughout adulthood for proper tissue function. We and others showed that loss of a single transcription factor, Chinmo, results in transdifferentiation of male somatic stem cells (CySCs) in the testis into the female "follicle-like" epithelial cells, leading to infertility (PMID: 25453558, 29389999). Additionally, we showed that *chinmo*-deficient-CySCs display ectopic expression of female sex determinants, Transformer (Tra)^{F} and Doublesex (Dsx)^{F}, and upregulate the epithelial morphogenesis program of ovarian follicle cells (PMID: 29389999;

33751104). However, it is still poorly understood how loss of Chinmo leads to the production of Tra^F. We obtained the transcriptome of WT and *chinmo*-mutant CySCs (PMID: 33751104). Bioinformatic analyses revealed that binding sites of Zelda (Zld), a pioneer transcription factor, were enriched in genes upregulated in *chinmo*-deficient CySCs. We confirmed that Zld protein is expressed in *chinmo*-deficient CySCs but is absent from WT CySCs. Fluorescent *in situ* hybridizations revealed that *zld* transcripts are expressed at similar levels in WT and *chinmo*-deficient CySCs. These data suggest that *zld* is post-transcriptionally modified during sex transformation. In support of this, we found that miRNAs present in WT CySCs degrade *zld* mRNA, as impaired activity of *dicer-1* – which is required for miRNA biogenesis – in WT CySCs causes a significant increase in Zld protein. We further showed that *zld* is critical for sex reversal because this process is significantly impeded when *zld* and *chinmo* are concomitantly depleted from CySCs. Zld overexpression in WT CySCs induced modest Tra^F production, but this was not sufficient to trigger sex reversal presumably because Chinmo and other male determinants are still expressed. Because Zld is unlikely to be an RNA-binding protein (RBP) that directly facilitates *tra* pre-mRNA alternative splicing in to tra^F, we searched for genes encoding RBPs that were Zld targets and were upregulated in *chinmo*-deficient CySCs: this yielded a list of six candidates, including Dp1. We showed that Dp1 protein is increased in *chinmo*-deficient CySCs and has a subcellular location similar to Dp1 in ovarian follicle cells. We are currently testing the hypothesis that Dp1 acts downstream to Zld and upstream of Tra^F in sex reversal. Our findings suggest a model in which a pioneer factor - Zld – alters the transcriptional landscape required for the cellular "reprogramming" of male CySCs into their female counterparts.

68 **Plasticity versus Paradigm: Follicle Stem Cell Identity shifts in response to environmental cues** Alana O'Reilly[1], Daniel Zinshteyn[2], Eric H Lee[2], Fred CC Miglo[3], Joseph CC Grosstephan[31]Cancer Signaling and Microenvironment, Fox Chase Cancer Center, [2]Molecular Therapeutics, Fox Chase Cancer Center, [3]Immersion Science Program, Fox Chase Cancer Center

Stem cells are critical for long-term tissue health. As such, they leverage mechanisms that enable rapid and robust response to environmental cues to ensure continuous generation of functional daughter cells throughout the lifetime of a tissue. Substantial effort has been focused on determining markers of stem cell "identity", based on long-standing paradigms that stem cells are a) stationary, b) self-renew via asymmetric cell division, and c) express molecular markers that are distinct from their daughters. In many tissues, this rigid concept of stemness has been challenged, with observations that multiple, distinct cell types accomplish stem cell function depending on environmental cues such as injury. Moreover, progenitor and differentiated cells can adopt a stem cell phenotype given the appropriate cues. The Follicle Stem Cell (FSC) field is currently rife with this type of controversy. Universally, investigators agree that FSCs are the anterior-most, lineage labeled cells of the ovarian follicular epithelium. More controversial is where, precisely, FSCs are located, which cells have the capacity to function as stem cells, and whether there is a distinguishing, unique molecular "identity" for FSCs. We took an agnostic approach to this issue, focusing first on changes in expression of the widely used molecular markers Eyes Absent (Eya) and Castor (Cas) within cells implicated as FSCs. We find dramatic changes in Eya and Cas protein expression in cells with FSC potential depending on feeding status, with adoption of nearly equal Eya-Cas signatures among distinct cell populations in nutrient-restricted flies. Going one step further, we mapped feeding-dependent transcription changes in FSCs using TU-tagging, which enables specific labeling of nascent transcripts within a specific cell population. Dynamic changes in both gene expression and enzymatic activity within FSCs were observed between starved and fed conditions, strongly supporting the idea FSC molecular identity shifts during adaptation to environmental conditions. Building on these observations, we are using our vast network of citizen scientists to conduct large scale image analyses aimed at defining the most robust and reliable parameters for FSC identification. This approach is unbiased, tests the utility of parameters posed as critical for FSC identification, and engages and includes participants worldwide in the science. Our results support the model that a plastic population of cells located across multiple cell layers within the stem cell compartment of the ovary can function as FSCs. We show that cells within this region shift identity and respond to diet-based environmental cues, inconsistent with the existence of a static, protected cell type with a set molecular identity.

69 **Chronic suboptimal temperatures impact spermatogenesis in Drosophila melanogaster** Ana Caroline Gandara[1,2], Daniela Drummond-Barbosa[1,21]University of Wisconsin-Madison, [2]Morgridge Institute for Research

Climate change have affected the abundance and distribution of many organisms around the globe. In this context, investigating how temperature impacts the reproduction of organisms is an important area of research. Insects are particularly sensitive to environmental temperatures. Gametogenesis is highly sensitive to environmental and physiological factors; however, it remains largely unknown how exposure of Drosophila melanogaster adults to chronic thermal stress affects gamete production. In a recent published study, we showed that exposure of females to 29°C

(warm) and 18°C (cold) temperatures decrease egg production through distinct effects. We also showed that 20-day exposure of males to 29°C decreases hatching rates, although the mechanisms of fertility reduction remained unknown. We therefore investigated how suboptimal temperatures affect spermatogenesis. In males maintained at 29°C (but not at 18°C), we observed that sperm abundance in seminal vesicles drastically decreases within 5 days. We also observed that the testes looked thinner at 29°C; so we counted the number of spermatids along the testes and observed that they were elevated at 18°C after 15 days but they were the same at 25°C and 29°C. Germline stem cell numbers in males at 29°C were comparable to those of control males over time (and increased at 18°C), suggesting that the reduced number of spermatids at 29°C might instead result from increased death. Interestingly, cold and warm temperatures lead to a significant increase in spermatid elongation/individualization defects; given the high sperm abundance at 18°C, these results indicate that sperm production has a high tolerance for errors during spermiogenesis. To measure the dynamics of fertility reduction at different temperatures, adult males from three different fly strains were maintained at 18°C, 25°C or 29°C over time. Male fertility was measured based on hatching rates of eggs laid by young females after mating. Hatching rates were significantly decreased after 10 days at 29°C (but not 18°C) relative to 25°C in all strains, consistent with the reduction of sperm abundance in seminal vesicles at 29°C. We are currently examining whether the ability of 29°C males to transfer sperm to females might also contribute to the reduced fertility. Altogether, our findings on how temperature affect male and female fertility in Drosophila are also relevant to warm-blooded animals, which are subject to hypothermia, heatstroke, and fever.

70 **The Head-Tail Connection Apparatus is a dynamic macromolecular structure that links the sperm tail to the haploid sperm nucleus** Kathleen H. M. Holmes, Brian J. Galletta, Nasser M. RusanNational Heart, Lung and Blood Institute, National Institutes of Health

The Head-Tail Connection Apparatus (HTCA) establishes and maintains the connection between a sperm head (nucleus) and tail (axoneme). From invertebrates to mammals, failure of the HTCA results in detachment of the sperm's genetic information from its transport machinery, which leads to male infertility. A key factor in forming the HTCA is the centriole, a microtubule based structure that acts as the physical link between the tail and head. A major question in the field revolves around the molecular composition and organization of the HTCA, yet very few factors have been linked to HTCA formation and function. We began our study by defining stage specific events that describe the major phases of HTCA *Establishment* and *Maintenance*. We have divided the *Establishment* phase into two stages: 1) *Search,* where the centriole is pulled into the nuclear envelope, and 2) *Attachment*, where the centriole and nucleus first interact. We have also subdivided the *Maintenance* phase into two stages: 1) *Embedding*, where the basal body physically moves into an invagination of the nuclear envelope, and 2) *Reorganization,* where the centriole is physically displaced to the side of the narrowing nucleus. We then determined the localization of known HTCA components at each of these stages using super resolution and expansion microscopy; effectively building a dynamic molecular map of HTCA proteins during sperm development. We found that Spag4, a SUN-domain protein, and Yuri Gagarin undergo significant remodeling throughout spermatid development, starting as a crescent during *Attachment*, forming a cap around the proximal end of the centriole during *Embedding*, and forming a previously undescribed "Shelf" perpendicular to the centriole and immediately above the Proximal-Like Centriole (PCL) during *Reorganization*. Interestingly, we also found a novel localization for the protein Uncoordinated (UNC) at this Shelf during *Reorganization.* The localization of these three proteins to the Shelf at the base of the nucleus and the top of the PCL, has lead us to propose a model whereby the PCL forms a ledge that guides the position of the nucleus as the centriole is repositioned. We are currently using this molecular map to understand how mutations in HTCA components affect each of our defined stages. So far, we have discovered that Spag4 and Yuri are not required for centriole establishment stages, but are required for the maintenance phase, which is contrary to the current model of their functions. Finally, to expand the list of HTCA molecular components, we performed a GFP-trap localization screen of candidate proteins and identified CG42399, TOGARAM1, as a novel HTCA protein. We are currently investigating the localization and function of TOGARAM1. Together our work serves as a critical foundation for our understanding HTCA formation and how its composition results in a stable, yet flexible connection between the sperm head and tail.

71 **Cohesion maintenance/rejuvenation during meiotic prophase depends on the chromatin remodeler Brahma and the mRNA-binding protein Pumilio, both of which have functional links to the cohesin loader, Nipped-B** Muhammad Abdul Haseeb[1], Alana C Bernys[2], Erin E Dickert[3], Sharon E Bickel[11]Biological Sciences, Dartmouth College, [2]Molecular Biology, Princeton University, [3]Pediatric Oncology, Dana-Farber Cancer Institute

Premature loss of sister chromatid cohesion during the decades-long prophase arrest of human oocytes contributes to the high incidence of aneuploid pregnancies in older women. We have discovered a cohesion rejuvenation program

that operates during meiotic prophase in Drosophila oocytes to establish new cohesive linkages and requires the cohesin loader, Nipped-B. Using a "tag-switch" reporter to express and monitor prophase-specific cohesin on meiotic chromosomes, we have found that chromosome-associated cohesin turns over extensively and that failure to load cohesin on chromosomes during meiotic prophase leads to arm cohesion defects. Moreover, knockdown of Nipped-B during meiotic prophase disrupts loading of prophase-specific cohesin on meiotic chromosome arms. To identify proteins required for cohesion rejuvenation, we performed a germline specific Gal4-UAS inducible RNAi screen using the prophase-specific mata-Gal4 driver. Two of the positives we uncovered, Brahma (Brm) and Pumilio (Pum), have functional links to the cohesin loader, Nipped-B. Brm is the ATPase subunit of two Drosophila chromatin remodeling complexes and the orthologous Swi/Snf complex in yeast interacts with the cohesin loader to promote its chromatin localization (Munoz et al 2019). Pum is a mRNA-binding protein that interacts directly with Nipped-B mRNA in ovary extracts (Gerber et al 2006). Our data indicate that knockdown of either Brm or Pum during prophase causes a significant increase in meiotic chromosome segregation errors, including those of recombinant homologs, consistent with premature loss of arm cohesion causing chiasmata destabilization in bivalents. Using fluorescence in situ hybridization to assay the state of cohesion directly, we have confirmed that knockdown of Brm or Pum during meiotic prophase causes premature loss of arm cohesion. In addition, using RNASeq, we have found that the abundance of Nipped-B transcripts is significantly decreased in Pum knockdown ovaries, consistent with Pum-dependent stabilization of Nipped-B mRNA. We hypothesize that Brm and Pum each promote cohesion rejuvenation during meiotic prophase by modulating Nipped-B activity. Tag-switch experiments are underway to determine whether cohesin loading onto meiotic chromosomes is disrupted when Brm or Pum is knocked down during meiotic prophase.

72 **Fasciclin-2 promotes anchoring of the polar cell processes that form the sperm-entry channel during micropyle morphogenesis** Kristin M Sherrard, Sally Horne-BadovinacMolecular Genetics & Cell Biology, University of Chicago

Dynamic rearrangements of epithelial cells play central roles in shaping tissues during development. There are also scenarios, however, in which epithelial morphogenesis synergizes with extracellular matrix (ECM) secretion to build complex acellular structures. A prime example of this epithelial craftsmanship is seen the Drosophila micropyle - the cone-shaped channel in the eggshell that acts as the sperm-entry point during fertilization. The eggshell is a multilayered ECM, consisting of vitelline membrane and chorionic layers, that is secreted by the follicular epithelial cells that surround the developing oocyte. To build the micropylar cone, ~40 of these cells evaginate from the oocyte surface while the two central-most cells, called polar cells, develop long processes that reach from their apical surfaces toward the oocyte. The evaginating cells secrete eggshell proteins into their central lumen to build the micropylar cone, the polar cell processes maintain the open channel through the middle of the cone for the sperm.
Electron microscopy studies performed by the Margaritis lab in the 1980s and 1990s showed that the polar cell processes are embedded in a thick plug of vitelline membrane with a dramatic maze-like structure that is distinct from the vitelline membrane around the rest of the oocyte. Thus, the open channel through the micropyle ends in a blind alley through which the sperm must penetrate to reach the egg. However, nothing is known about how the polar cell processes form nor how this region of vitelline membrane attains its unusual structure. To address these questions, we performed a time course of polar cell process development using 3D reconstructions from confocal images and did a candidate-based RNAi screen to identify key molecular players. We found that the apical surfaces of the polar cells associate with the vitelline membrane from the earliest stages of micropyle formation and that the processes lengthen through a stretching mechanism as the polar cell bodies are pushed away from this anchor point by the evaginating cells. We further found that Fasciclin-2 is highly expressed on the process termini where it plays a role in anchoring them to the vitelline membrane. We are now testing the hypothesis that the polar cell processes additionally help create the maze-like pattern in this ECM, such that the cells and vitelline membrane progressively shape one another's structure.

73 **An intruder-targeting system eliminates paternal mitochondria after fertilization in *Drosophila*** Sharon Ben-Hur[1], Sara Afar[1], Keren Yacobi-Sharon[1], Yoav Politi[1], Liron Gal[1], Ofra Golani[1], Rebecca Haffner-Krausz[1], Elina Maizenberg[1], Dorit Kalo[2], Zvi Roth[2], Eli Arama[11]Weizmann Institute of Science, [2]The Hebrew University of Jerusalem

Maternal inheritance of mitochondria occurs in almost all organisms, spanning from fungi and plants to humans. Upon fertilization, a single sperm fully penetrates the egg, resulting in a short period where mitochondria from both gametes populate the early embryo. Within a few cell cycles, however, paternal mitochondria (**PM**) are eliminated, and propagating maternal mitochondria take over the mitochondrial network. Several reports illustrate PM elimination as an outcome of passive dilution by the vast maternal mitochondrial pool. Yet, recent studies, performed on different organisms, suggest active elimination of PM by egg-derived mechanisms. In particular, our group previously

demonstrated that egg-derived multi-vesicular bodies (**MVBs**) associate with PM immediately after fertilization to promote PM degradation. Nevertheless, the mechanisms by which MVBs mediate paternal mitochondrial destruction remain unknown[1].

Here, I will present our recent findings aiming to identify the mechanisms by which egg-derived MVBs target and destroy PM in *Drosophila*. We identify a cell intruder-targeting pathway, called LC3-associated phagocytosis (LAP), which is a common endocytic, autophagic and phagocytic pathway, as the main executer of PM elimination. Our model indicates that MVBs loaded with LAP-specific components engage with PM to mediate its elimination. LAP-specific phosphatidylinositol 3-phosphate kinase (PI3K) complex is recruited to PM for PI3P production. The presence of PI3P, together with generation of ROS, promote Atg8 (LC3) conjugation to PM, facilitating sequestration of PM to lysosomes. Finally, I will also present initial evidence for possible conservation of some of these mechanisms during corresponding processes in mammalian eggs.

1. Politi, Y. et al. Paternal mitochondrial destruction after fertilization is mediated by a common endocytic and autophagic pathway in Drosophila. Dev. Cell 29, 305–320 (2014).

74 **A centromere-encoded retroelement generates transcripts that localize to centromeres in *cis*** Bryce Santinello[1], Ruiyi Sun[1], Laura Leo[1,2], Asna Amjad[1], Barbara Mellone[3,41]Molecular and Cell Biology, University of Connecticut, [2]Genetics, Universita' La Sapienza, [3]University of Connecticut, [4]Institute for Systems Genomics, University of Connecticut

Centromeres are essential chromosomal landmarks that dictate the point of attachment between chromosomes and spindle microtubules during cell division. The centromeres of *D. melanogaster* consist of islands of complex DNA enriched in retroelements flanked by arrays of simple satellites. While each centromere contains different assortments of DNA elements, all of which are also present elsewhere in the genome, only one element, the non-LTR retroelement *Jockey-3,* is shared amongst all centromeres. *Jockey-3* is enriched at centromeres compared to the rest of the genome and is a conserved centromere component in *D. simulans,* a species that diverged from *D. melanogaster* 2.5 million years ago and that contains highly divergent centromeric satellite DNA. Retroelements are enriched at the centromeres of organisms across taxa, but the significance of this association remains unclear. Retroelements have been proposed to facilitate the transcription required for the maintenance and propagation of the unique chromatin that marks the centromere, or to produce non-coding RNAs with a structural role at the centromere. Here, we investigate the transcription and subcellular localization of *Jockey-3* transcripts. Using single-molecule RNA FISH combined with immunofluorescence, we detect *Jockey-3* at all centromeres in mitosis and interphase in three different cell types. Taking advantage of the polymorphism of *Jockey-3*, we show that these transcripts remain associated with their cognate sequences in *cis*. Using a targeted ectopic centromere system, we show that *Jockey-3* RNAs do not localize at *de novo* centromeres, which would be expected if they played a structural role. We also test if inhibition of nascent *Jockey-3* transcription affects centromere integrity and if centromeric proteins are required for *Jockey-3* transcription and RNA localization. Together, this work sheds light on the functional significance of this conserved retroelement at *D. melanogaster* centromeres, supporting a model where its nascent transcription, rather than a structural role, contributes to centromere identity.

75 **HP1 Interacts with the Chromosomal Passenger Complex to Promote Spindle Assembly and Chromosome Segregation** Siwen Wu[1], Kim McKim[21]Rutgers University, [2]Waksman Institute, Rutgers University

Accurate chromosome segregation during female meiosis is essential for genome integrity and errors in this process can lead to infertility, miscarriages, and catastrophic birth defects. The highly conserved chromosomal passenger complex (CPC) serves as a "master regulator" of spindle assembly and ensures accurate bi-oriental chromosome segregation during meiotic cell division. The CPC is composed of 4 subunits: the inner centromere protein (INCENP), Borealin, Survivin and Aurora B kinase. The CPC regulates meiotic events through its dynamic changes in localization at kinetochores and the central spindle. It recruits microtubules to the chromosomes for acentrosomal meiotic spindles and regulates kinetochore-microtubule attachments for accurate chromosomes segregation in meiosis 1. However, the mechanism responsible for the CPC recruitment to the chromosomes and the interaction between the CPC and spindle microtubules is unknown. Loss of *Drosophila* INCENP or Borealin leads to failures in chromosome-microtubule attachment, spindle assembly and chromosome segregation. INCENP and Borealin both contain Heterochromatin protein-1 (HP1) binding domains. HP1 is a chromatin protein mediating heterochromatin formation, chromatin packaging and gene expression. Our previous study suggested that HP1 can be a potential binding site for the CPC to

target heterochromatin. We previously generated two mutants deleting the two INCENP microtubule binding domains in *Drosophila* oocytes. These mutants showed weak spindle defects, revealing a more complicated regulatory system in spindle assembly. We then generated a mutant that deleted C-terminal domain of Borealin from a *borr:Incenp* fusion gene and all HP1 binding sites on both Borealin and INCENP. This mutant showed severe spindle defects, indicating a crucial role of the CPC-HP1 interaction in targeting the CPC to the chromosomes. Therefore, we predicted that HP1 recruits the CPC to the chromosomes to promote spindle assembly and chromosome segregation. To better elucidate the interaction between HP1, the CPC and meiotic chromosomes, we generated genetic models disrupting HP1 in oocytes by employing RNAi and a degron system. We are examining the phenotypes in these mutants by investigating 1) the localization of HP1, the CPC and centromeres on metaphase chromosomes, 2) spindle defects, and 3) chromosome alignment through confocal microscopy. Parallel to these studies, we are generating an INCENP mutant with HP1 binding domain deletion to assess the defects in spindle morphology and chromosome mis-segregation. Collectively, dissecting on how CPC-HP1 interaction promotes bipolar spindle organization and accurate meiotic chromosome segregation will provide better insight into the fundamental mechanism of female meiosis.

76 **Visualization of CRISPR/Cas9 Induced Mitotic Crossovers Suggest Multiple Types of double Holliday Junction Resolution** Evan Dewey[1], Jeff Sekelsky[21]Genetics, University of North Carolina-Chapel Hill, [2]Genetics, University of North Carolina at Chapel Hill

Genome stability is key to longevity of multicellular organisms and avoidance of disease. Despite daily challenges from numerous sources of DNA damage threatening this stability, cells regularly repair DNA to maintain genomic resilience. Improper or misregulated repair causes accumulation of detrimental mutations within the genome, eventually leading to genome instability, cancer, and other genetic disease. Homology directed repair (HDR) of DNA double strand breaks is one DNA repair pathway that, if improperly regulated, leads to accumulation of mutations via mitotic (non-meiotic) crossovers and loss of heterozygosity. Therefore, better understanding of mitotic crossover mechanisms and regulators is critical to prevention of cancer and other genetic disease. CRISPR/Cas9 has also become increasingly reliant on accurate HDR, specifically repair that does not result in crossovers, to integrate desired mutations or corrections in genome editing. Precise interpretation of mitotic crossover mechanisms relies on the presence of SNPs within crossover product heteroduplex DNA, DNA consisting of sequence from both the repaired and template DNA molecules. Mitotic crossover mechanisms in multicellular organisms remain elusive however, as SNP mismatches within heteroduplex DNA are obscured through repair by mismatch repair (MMR). Through mismatch repair (MMR) knockout only possible in *Drosophila* (through both *Msh6* canonical and backup *Xpc* short-patch pathways), it is now possible to visualize heteroduplex DNA in resulting mitotic crossover products using Sanger sequencing for CRISPR/Cas9 induced double strand breaks. Using this unique *Drosophila* tool, I have reconstructed several independent mitotic crossover events and identified mechanisms from breaks induced by CRISPR/Cas9. Both hypothesized forms of double Holliday Junction resolutions are indicated by the arrangement of homoduplex and heteroduplex DNA I have observed, though the amount of heteroduplex DNA is variable on either side (5’ or 3’) of the crossover, indicating that there may be a preference for synthesis in a certain direction. I hypothesize this preference is caused by the strand (e.g. + or -) to which Cas9 is bound, as it may inhibit that strand from invading the template molecule. This work has enhanced understanding of how DNA is repaired by HDR, expanding knowledge of how mitotic crossovers lead to genome instability and providing better understanding of how to beneficially utilize mitotic crossover mechanisms in CRISPR/Cas9 genome editing.

77 **The Regulation of Prospero by miR-190 during asymmetric cell division in *Drosophila* Neuroblasts** Gerson Ascencio, Blake RiggsBiology, 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 aPKC, a protein kinase enzyme, Prospero, a transcription factor, and Numb, a membrane-associated inhibitor of Notch signaling. These determinants are partitioned asymmetrically to drive cell fate selection, with Prospero moving towards the basal cortex to promote cell differentiation at 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.

78 **Loss of Spargel/dPGC-1 function in larval endoreplicating tissues negatively influence metabolism and growth** Mohammed Shah Jalal, Atanu DuttaroyBiology, Howard University

In mammals, the PGC-1 (Peroxisome proliferator-activated receptor-gamma coactivator-1) transcriptional co-activator serves as a master regulator of energy metabolism and a robust activator of mitochondrial biogenesis. An ancestral PGC-1 homolog appears in Drosophila called Spargel/dPGC-1 (*srl*), which is essential for ovarian growth as germline-specific knockdown of *srl* halts egg chamber development, resulting in arrested oogenesis and female sterility. Recent isolation of a *srl* deletion (srl^{del}) turned out to be an amorphic allele (srl^{null}) for the gene that dies unhatched inside the eggshell at the late embryonic/early larval boundary. Metabolomic profiles of the *spargel* -deficient embryos revealed *srl* mutant embryos fail to metabolize carbohydrates most likely because TCA cycle enzymes are depleted in srl^{null} mutant. Therefore, inability to fulfill energy demand appears to be the root cause for embryonic lethality of srl^{null} mutants. As a transcriptional co-activator, Spargel expression is limited to the nuclei of the ovary. Now we claim that *spargel* expression is dynamics, because, in most larval tissues, Spargel is expressed in the cytoplasm. 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 can be real since the global phosphoproteome profile of Drosophila embryos identified Spargel as a candidate for phosphorylation. With the help of a phospho-specific Spargel Ab raised in the lab we now confirmed the phosphorylation status of cytoplasmic Spargel. 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 normal nuclear size. Thus, our findings support that during larval growth Spargel plays an essential role in endoreplicating tissues.

79 **Local Ecdysone Activation Enforces Robust Cell Cycle Exit in the Pupal Eye** Elizabeth Fogarty, Laura ButtittaMolecular, Cellular, and Developmental Biology, University of Michigan

During development of the compound eye in *Drosophila melanogaster*, cellular proliferation is temporally coordinated with specification and differentiation to give rise to a highly ordered structure of the correct size and architecture. Previous studies have delineated cell intrinsic factors and extracellular signaling pathways that are important for the highly regulated cell cycle dynamics in the larval eye disc. Less well studied are the processes controlling proliferation in the pupal eye, where unspecified cells continue dividing until approximately 24 hours after puparium formation. This cell cycle exit is temporally associated with the completion of cell patterning and initiation of terminal differentiation; however, how the cycling status and differentiation status of the tissue are coordinated with one another during metamorphosis is not well understood. Our group has previously described a paradigm of cell cycle exit during metamorphosis comprising a flexible phase, where expression of a single cell cycle regulator is capable of driving ectopic divisions, followed by robust cell cycle exit, after which multiple regulators must be introduced to support continued cycling. Here, we present new genetic models where we cause a delay (with CycE) or a bypass (with CycE and CycD/Cdk4) of cell cycle exit in the pupal eye. Using immunofluorescent markers and RNA-Seq in these models, we show that ectopic proliferation impinges on the differentiation programs in multiple cell types. These analyses reveal that bypass of cell cycle exit is associated with compromised induction of the ecdysone transcriptional cascade, and that this block in signaling is correlated with failure to express shade (shd), the enzyme responsible for local prohormone activation. To determine the relevance of these findings and test the ability of shd to modulate proliferation and/or differentiation in the pupal eye, we overexpressed shd in the delay and bypass models described above. Strikingly, we find that shd expression does not alter proliferation in these models during the flexible phase of cell cycle exit, but that shd completely abolishes cycling in the bypass model after the robust cell cycle exit timepoint. Taken together, our findings contradict previous reports that cycling status and differentiation programs are fully separable and suggest that the ecdysone signaling cascade is capable of enforcing robust cell cycle exit during metamorphosis.

80 **Elimination pathways of spontaneous neoplastic mutant clones during development** Hojun Jeon, Marine

Stefanutti, Allison BardinInstitut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France

Tumor incidence is believed to correlate with the risk of somatic mutations in a given tissue, however, the extent to which cell elimination pathways limit tumor initiation is not fully understood. In particular, the role of cell-cell competition or cell-autonomous elimination mechanisms in this process remains to be fully investigated. Here, we establish genetic methods to detect and measure spontaneous mutation frequency of neoplastic clones during development *in vivo.* We take advantage of a null mutant allele for two adjacent paralogs, *polyhomeotic distal* (*ph-d*) and *polyhomeotic proximal* (*ph-p*), which are X-linked tumor suppressor genes. Spontaneous *polyhomeotic* (*ph*)-deficient clones were sporadically observed in 3rd instar larval discs of heterozygous mutant females but not in wild-type males suggesting mitotic recombination as a prominent mutation mechanism. Our data suggest that most spontaneous mutations arise late in development (72-96h after egg laying), when there is the largest number of progenitor cells. Blocking apoptosis by p35 expression either ubiquitously or clonally did not alter the frequency of spontaneous mutant clones. Thus, these data argue against a role for classic cell competition pathways in eliminating spontaneous neoplastic mutant clones. In contrast, p53-knockdown significantly increased the frequency of spontaneous *ph*-deficient clones suggesting that a p53-associated elimination pathway limits the appearance of neoplastic mutant clones independent of its role in apoptosis. Moreover, we provide evidence that reducing cell delamination through knockdown of *slit* increased the appearance of *ph*-deficient clones. Our findings thus give insight into mechanisms limiting spontaneous neoplastic clone development. We are currently testing a working model in which different elimination pathways may act at distinct times in development of a neoplastic growth, with p53 restraining initial appearance of a mutant clone, and cell delamination impacting the ability of the neoplastic clone to remain in the tissue.

81 **Beyond basic: pH dependent mechanisms of brain development** Bernice Lin, Isabella Maag, Beverly J. PiggottDivision of Biological Sciences, University of Montana

The regulation of pH is critical to all biochemical processes and cellular viability. The cell-specific role of pH in developmental processes is poorly defined. Na+/H+ exchangers (Nhe) are major regulators of intracellular pH. They are broadly expressed, yet evidence suggests that their relative expression levels drive pH set points that facilitate specialized cell-type specific functions in neural development. This is evidenced by the fact that mutations in Nhe proteins cause neurodevelopmental disorders including microcephaly, autism spectrum disorders, epilepsies, and Christianson syndrome. Despite the clear importance of Nhe proteins in brain formation, their role in developmental processes is not well understood. Brain development requires the coordinated actions of neural stem cells to temporally generate enough neurons with the correct identities. Glial cells play important roles supporting neural stem cell proliferation and developmental transitions. Studies in *Drosophila* have provided insight into the symbiotic relationship between glia and neural stem cells (called Neuroblasts in the fly) through developmental transitions. Glial cells facilitate stem cell reactivation from quiescence and support of stem cell self-renewal. Our preliminary work examined a genetically encoded pH sensor in developing larval brain and found dynamic pH changes across the brain. To investigate the importance of pH in brain development we knocked down Nhe proteins in either glia or neuroblast lineages and found they both caused a major reduction in brain size. A possible explanation for a smaller brain could be compromised proliferation. Nhe knockdown in neuroblasts or glia similarly reduced the number of proliferating cells. Nhe proteins regulate pH, but they also have been found to play a major role in cellular swelling during mitosis. The mitotic swelling has been attributed to Nhe mediated Na+ influx. If Nhe cells are important for cell division, we would expect that neuroblasts would exhibit a more basic pH (due to H+ efflux of these exchangers) compared to their more differentiated progeny. Indeed, examining pH sensors within the type II neuroblast lineages revealed neuroblasts were more basic than their progeny. In glia – upon Nhe knockdown we found a significant reduction in proliferating Repo+ (glial marker) cells indicating that Nhe proteins are also important mediators of gliogenesis. Proliferating glial cells are a minor component of proliferating cells in the fly brain compared to neural progenitors. Thus, it's likely that Nhe manipulation also compromises glial support of the neuroblast niche. Our ongoing experiments seek to define the role of pH in glia and neuroblasts during brain development. This work will reveal pH-sensitive mechanisms of development and investigate why specific pH set points support proliferative vs. differentiated cellular programs.

82 **The circular RNA *circ_R41* regulates anti-viral immunity and ROS production in *Drosophila*** Weihong Liang[1], Wei Liu[1], Xiao-Peng Xiong[2], Jian-Liang Li[3], Rui Zhou[11]Johns Hopkins University School of Medicine, Johns Hopkins All Children's Hospital, [2]Sanford Burnham Prebys Medical Discovery Institute, [3]National Institute of Environmental Health Sciences

Non-coding RNAs (ncRNAs) play a key role in modulating host-virus interactions. For example, small interfering RNAs can guide the RNA interference machinery to target viral RNAs for destruction in a wide variety of organisms. In addition, microRNAs can profoundly impact the magnitude and duration of the inflammatory response in response to viral infection by targeting mRNAs encoding signaling proteins or inflammatory cytokines. Furthermore, select ncRNAs (e.g., EBERs and HSURs) can modulate the viral life cycle. Recently circular RNAs (circRNAs), which are products of "head-to-tail" back-splicing events, have been discovered in diverse eukaryotes, and constitute the latest addition to the ncRNA collection. Select circRNAs have been shown to sequester PKR and modulate anti-viral immunity in mammals.

We have identified and validated a collection of circular RNAs in *Drosophila melanogaster*. We show that depletion of the circular RNA *circ_R41*, but not its linear sibling, compromises replication of a panel of RNA viruses, including Flock House virus, *Drosophila* C virus and Cricket paralysis virus, in cultured *Drosophila* cells. Similarly, depletion of *circ_R41 in vivo* reduces viral load and enhances host survival upon RNA virus infection. Importantly, restoring *circ_R41* expression in *circ_R41*-depleted cells or flies suppresses the viral replication and/or host survival phenotypes. In addition, our analyses reveal that *circ_R41* is enriched in the fly gut tissue, and that gut-specific depletion of *circ_R41* compromises viral replication *in vivo* in an oral infection model. Furthermore, we find that defects in RNA virus replication in *circ_R41*-deleted flies correlate with increased levels of reactive oxygen species (ROS) and enhanced expression of *DUOX* (*Dual oxidase*), which is involved in ROS biogenesis. Notably, *circ_R41* and DUOX associate with each other. Lastly, we show that feeding flies with N-acetyl-L-cysteine, a ROS inhibitor, depletion of *DUOX*, or overexpression of *IRC* (Immune-regulated catalase), which removes ROS, leads to a reduction in ROS levels and suppresses the viral replication defects elicited by *circ_R41* depletion. We conclude that *circ_R41* regulates anti-viral immunity, at least in part, by modulating ROS production.

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83 **The DNA Damage Response regulates epithelial cell dynamics in the infected gut** Peter Nagy[1], Bretta Hixson[2], Nicolas Buchon[21]Entomology, Cornell University, [2]Cornell University

Intestinal microbes are central to host health but can also constitute an important etiological factor for the initiation and progression of diseases. However, the mechanisms by which pathogenic microbes influence intestinal tissue health remain largely unknown. Our overarching goal is to understand how pathogenic microbes inflict stress on the intestinal epithelium in *Drosophila melanogaster* and to characterize the impact of the molecular dialogue on cell dynamics. This is particularly important in the gut tissue where intestinal barrier, immune and digestive functions need to be coordinated with cell turnover. We and others have demonstrated that microbes stimulate epithelial turnover and initiate a feedback loop in which the elimination of enterocytes (ECs) activates intestinal stem cell proliferation in order to replace stressed/dying cells, balancing cell loss with tissue renewal. However, the molecular mechanisms that lead to EC loss in response to infection or innate immune activation are still not understood. We have found that infection induces an immediate host response in a subpopulation of midgut ECs which accumulate the DNA damage histone mark, gH2av (the phosphorylated form of H2av). Importantly, we have shown that pathogens, but not the commensal microbiota, activate the DNA damage repair (DDR) pathway which modulates epithelial cell elimination, cytokine production and stem cell proliferation. Functional/bacterial genetics and microscopy imaging techniques allowed us to determine the microbial characteristics and epithelial cell state that influence DDR pathway activation. Gut transcriptome analyses and cell-tracing methods previously developed in our lab enable us to identify how DDR pathway activation influences both cell elimination and pro-survival pathways. This work expands our understanding of how extrinsic factors and intrinsic cellular processes predispose cells to stress inflicted by infection-induced damage. Our study demonstrates that DDR pathway activation is a novel signaling route modulating cell loss and cytokine production, which is critical for health and homeostasis in a high-turnover tissue such as the intestine.

84 **Molecular and transcriptional characterization of a symbiotic niche mediating gut microbiome colonization in *Drosophila melanogaster*** Haolong Zhu[1,2], Allan Spradling[1,2,3], William Ludington[1,21]CMDB program, Johns Hopkins University, [2]Embryology, Carnegie Institution for Science, [3]Howard Hughes Medical Institute

The gut microbiome has been recognized to be crucially important for host physiology. However, the diversity of intestinal bacterial species and the presence of irrelevant transient bacteria have made it difficult to characterize molecular mechanisms governing specific microbiome functions. We believe that focusing on species interactions, which are sufficient to maintain stable, site-specific host associations, i.e. symbiotic niches, provides a feasible solution to

this challenge. Our previous work showed that such a niche exists in the *Drosophila* foregut that specifically promotes an association with *Lactiplantibacillus plantarum* (*Lp*WF). Here, we have characterized the cellular structure and gene expression of the niche region to determine its molecular components and physiological functions.

Our studies provide views of the niche at increasingly higher levels of resolution. Microscopic analyses revealed a specific *Lp*WF-foregut interaction at a distinctive extracellular layer that likely constitutes a niche substrate for *Lp*WF binding. This layer arises as a secretion from host cells near or directly within the niche. To identify the responsible cells, we conducted single-cell RNA sequencing on the *Drosophila* foregut in triplicate where we captured more than 10,000 cells per library. We assigned cell type identities to the clusters by examining hundreds of transgenetic *in vivo* reporter lines corresponding to cluster-specific genes, a few of which are located at the core *Lp*WF-binding-site of the niche. This, combined with our bulk RNA sequencing of the putative niche secretory cells, led us to identify a small group of abundantly and specifically expressed secretory gene candidates that might constitute or control the production of the niche substrate. We are currently evaluating the candidates to test their necessity and sufficiency for colonization specificity using an *in vivo* colonization assay with fluorescently labeled *Lp*WF.

To delineate the physiological functions of the niche, we characterized the transcriptional identities of the individual cell types within the *Drosophila* foregut region, which includes the proventriculus, the crop, the esophagus, the adult salivary gland, the corpus allatum, and the corpus cardiacum. Our data suggest that the *Drosophila* foregut strikingly resembles the human anterior digestive tract, i.e. the mouth, the esophagus, the salivary gland, the pituitary gland, and the tonsils. This evolutionary conservation manifests the potential of establishing the *Drosophila* foregut model to investigate the human anterior digestive tract at homeostasis and in diseases.

85 **Gut derived cytokine signalling mediates hypoxia tolerance** Kate Ding[1,2], Byoungchun Lee[1,2], Danielle Polan[3], Savraj Grewal[1,21]Biochemistry and Molecular Biology, University of Calgary, [2]Arnie Charbonneau Cancer Institute, [3]University of Calgary

Our cells and organs need oxygen in order to function. However, in some pathological conditions such as stroke, heart disease and cancer, our tissues are deprived of oxygen. This lack of oxygen, known as hypoxia, leads to the tissue damage and deregulation that characterizes these diseases. Understanding how tissues respond to low oxygen is therefore an important question in health research. While extensive studies have identified hypoxic responses in cell culture, they leave open the question of how tissues and whole organisms deal with hypoxia. This is important since tissue-to-tissue crosstalk often underlies hypoxic responses in animals.

In their natural ecology, *Drosophila* have evolved to grow on rotting, fermenting food rich in microorganisms – an environment characterized by low ambient oxygen. Hence, they provide a robust genetic model system to study how hypoxia influences physiology. Here we describe a mechanism of hypoxia tolerance in *Drosophila* involving the cytokine Unpaired 3 (Upd3), the fly interleukin-6 (IL-6) homolog. We found that expression of *upd3* is strongly upregulated in multiple tissues in adult flies exposed to hypoxia (1% oxygen). We found that *upd3* whole-animal null mutants show reduced survival in hypoxia, indicating that *upd3* signaling is required for hypoxia tolerance. Using tissue-specific RNAi-mediated knockdown, we have shown that these survival effects require *upd3* production in the enterocytes of the fly intestine. Interestingly, we also found that fat body knockdown of HIF-1 alpha, the classic hypoxia-induced factor required for low oxygen survival, lead to a further enhancement of intestinal *upd3* levels in hypoxia. When we genetically mimicked this enhancement, we found animals also had reduced survival in low oxygen. These findings suggest *upd3* signaling must be tightly regulated in hypoxia; induction of *upd3* is required to mediate survival in low oxygen, but excessive *upd3* production can lead to a 'cytokine-storm'-like response that contributes to lethality. Previous studies have demonstrated a link between HIF-1α and immune system modulation, thereby already implicating a role of hypoxia in immunity. However, with our discovery that gut-derived *upd3* is modulated in hypoxia and may be regulated in part by fat body HIF-1α, we have uncovered a role for tissue-to-tissue communication in mediating hypoxia tolerance.

86 ***Wolbachia* confers olfactory changes through the modification of glutamic acid decarboxylase (GAD) in *Drosophila melanogaster*** Sarah M Boothman, Jonathan MindenBiological Sciences, Carnegie Mellon University

Infection with parasites and intracellular microbes can lead to behavioral changes within the host. The "Behavioral Manipulation Hypothesis" claims these pathogens evolved the ability to modulate these behaviors to facilitate successful transmission (Thomas et al., 2005). However, there is much debate over the validity of this hypothesis, prompting the need for in-depth studies of mechanisms underlying microbe manipulation of host behavior. We use *Drosophila melanogaster* and its obligate intracellular symbiont *Wolbachia pipientis* as a model for this phenomenon, as multiple

host effects have been observed upon infection, including changes in behavior, mating, and fitness (Goodacre & Martin, 2012; Bi & Wang, 2019). In our work, we specifically investigate the effect of *Wolbachia* infection on olfactory perception of food odors. Using an odor-baited trap assay, we have shown that *Wolbachia*-infected flies have an improved response to yeast odors compared to their uninfected counterparts. To uncover the mechanism by which *Wolbachia* confers this change, we compared the protein profiles of infected and uninfected fly heads via two-dimensional difference gel electrophoresis (2D-DIGE). Interestingly, we observed a post-translational modification (PTM) of glutamic acid decarboxylase (GAD) upon infection. Given this enzyme's role in GABA synthesis, we next investigated how changes in GABA signaling could lead to altered olfactory responses. We perturbed GABA signaling using pharmacological treatments in our odor trap assays, and the results of these treatments suggest that increased GABA signaling leads to an improvement of olfactory perception in *Drosophila*. Thus, we propose a model in which *Wolbachia* infection leads to a PTM on GAD which increases GABA signaling and results in improved perception of food odors. Modification and regulation of GAD is largely understudied in *Drosophila*, so our current work aims to identify the type of PTM conferred upon infection and discover how it may contribute to the observed change in olfactory behavior. The results of this work will provide insight into the molecular mechanisms by which *Wolbachia* modulates *Drosophila* biology to better understand how pathogens manipulate host behavior more broadly.

87 **CRISPR screens in *Drosophila* cells identify Vsg as a Tc toxin receptor** Ying Xu[1], Raghuvir Viswanatha[2], Oleg Sitsel[3], Daniel Roderer[4], Haifang Zhao[5], Christopher Ashwood[6], Cecilia Voelcker[6], Songhai Tian[7], Stefan Raunser[8], Norbert Perrimon[2], Min C Dong[11]Urology, Boston Children's Hospital, [2]Genetics, Harvard Medical School, [3]Max Plank Institute, [4]Leibniz-Forschungsinstitut für Molekulare Pharmakologie, [5]Xuzhou Medical University, [6]Beth Israel Deaconess Medical Center Glycomics Core, [7]Boston Children's Hospital, [8]Structural Biochemistry, Max Planck Institute

Genome-wide CRISPR knockout screening harnesses the precision of CRISPR genome editing to perform genome-scale functional genomics, which is now a staple of cell biology and host-pathogen interactions studies in mammalian cells. Here, a pool of millions of Cas9-expressing cells is transfected such that each cell contains one of tens of thousands of different CRISPR guide sequences. The pool is then subjected to a perturbation, and the change in the distribution of each guide is measured through next-generation sequencing. We show that this technology can be made to work in Drosophila S2R+ cells. Next, we asked whether this method could be used to reveal the entry pathway of an insect toxin. Toxin complex (Tc) toxins are major virulence factors of bacteria such as *Photorhabdus luminescens* bacteria, which are deployed by entomogenous nematodes as a weapon to subdue and prey upon insect larvae. No protein receptors are known for any Tc toxins, limiting our understanding of their specificity and pathogenesis. We used genome-wide CRISPR knockout screening in flyS2R+ cells to identify Visgun (Vsg) as a receptor for an archetypal *P. luminescens* Tc toxin (pTc), which recognizes the extracellular *O*-glycosylated mucin-like domain of Vsg containing high density repeats of proline, threonine, and serine (HD-PTS). Vsg orthologs in mosquitoes and beetles contain HD-PTS and can function as pTc receptors, whereas orthologs without HD-PTS such as moth and human versions are not pTc receptors. Consistently, Vsg knockout flies showed reduced bacterial loads and lethality from *P. luminescens* infection. Our findings identify a proteinaceous Tc toxin receptor, reveal how Tc toxins contribute to *P. luminescens* pathogenesis, and establish a genome-wide CRISPR screening approach for investigating insecticidal toxins and pathogens.

88 **Chronic Infection, Imperfectness of Pathogen Detection, and the Evolution of Adaptive Suicide.** Peter V Lidsky, Jing Yuan, Raul AndinoMicrobiology and Immunology, University of California San Francisco

The interactions between infection, immunity, and aging are poorly understood. Immunosenescence, or age-related immune function deregulation, has attracted recent attention because of higher mortality in the elderly population during the ongoing COVID-19 crisis. In turn, some infections can modulate aging: for example, it is known that HIV and HCV chronic infections would cause accelerated age advancement in patients. We investigate these effects' molecular mechanisms and ecological roles using experimental and theoretical approaches. Here we report our results on immunosenescence and accelerated aging in the Drosophila system. To determine whether immunosenescence manifests in decreased tolerance (the ability of the host to withstand pathogenic effects), or decreased resistance (the host's ability to eliminate pathogens), we analyzed virus infections in young and old flies. Paradoxically, our data suggested an age-related decrease in the organism's tolerance or an increase in resistance to disease. To investigate effects of infection on the speed of aging, we introduced virus replicon expression in flies and found that animals with infection mimetic die faster than their control counterparts. Using transcriptomic aging clocks, we demonstrated that elevated mortality is accompanied by acceleration of aging. Finally, we rationalize our findings from an evolutionary perspective with mathematical modeling of epidemics. We have constructed a comprehensive theory of adaptive suicide that can explain when hosts might sacrifice their fitness to protect their kin from harmful infections. Our model can

encompass the infection-induced adaptive suicide in yeast and bacteria, limited lifespan in higher lifeforms, acceleration of aging by infection, and changes in tolerance and resistance with age.

89 **Identifying of Enhancers of the *Drosophila* Innate Immune System** Lianne Cohen[1], Zeba B Wunderlich[21]Biology, Boston University, [2]Boston University

In response to microbial infection, *Drosophila melanogaster* launches a transcriptional response to defend against pathogens and protect the fly. The two major immune pathways, Toll and IMD, coordinate the detection of microbes and ultimately activate their respective transcription factors, Dif and Relish. These transcription factors then interact with regulatory DNA, specifically promoters and enhancers. Enhancers are *cis*-regulatory sequences containing transcription factor binding sites (TFBS) that can direct gene expression at a distance along the chromosome from the transcriptional start site. While enhancers and TFBS have been identified for several immune responsive genes in *Drosophila*, the vast majority of enhancers that regulate immune induced genes are unknown and uncharacterized. In order to further study and identify immune responsive enhancers, we first established an immune model in cell culture by optimizing induction in S2 cells for the Toll and IMD pathways, as well as both pathways simultaneously. Then we employed STARR-seq (Self Transcribing Active Regulatory-Region sequencing), an activity-based assay which identifies sequences that can drive their own expression upon activation of immune signaling pathways in a high-throughput manner. We performed a pilot screen focused on regions surrounding known immune induced genes, totaling around 2% of the *D. melanogaster* genome. We confirmed enhancer activity previously described upstream of the antimicrobial peptides, Diptericin B and Attacin D. Furthermore, we identified novel immune specific enhancers surrounding other genes, for example CrebA, a transcription factor required for immune gene expression. We are additionally performing STARR-seq genome-wide, to identify immune responsive enhancers independent of their proximity to known immune genes. This systematic identification of enhancers throughout the genome allows us characterize the regulatory structure found in these sequences, as well as unknown immune TFBS. The identification of immune enhancers will provide insights into how animals use regulatory sequences control their gene expression response not only to pathogenic infections, but also to other rapidly changing environmental stimuli.

90 **That's how they roll, the motor pattern of rolling escape locomotion in *Drosophila* larvae** Liping He, Lydia Borjon, W. Daniel TRACEYIndiana University

When undisturbed, *Drosophila* larvae move forward through their environment with sweeping waves of caudal to rostral muscle contraction. In stark contrast, nociceptive sensory stimuli (such as attacks by parasitoid wasps) trigger the larvae to roll across the substrate by corkscrewing around the long body axis. While studies have described the motor pattern of larval crawling, the motor pattern of larval rolling escape locomotion remains unknown. Here, we have determined this pattern. To do so, we developed a high speed confocal time-lapse imaging preparation that allowed us to trigger rolling with optogenetics while simultaneously imaging a genetically encoded calcium sensor that was expressed in the muscles. Of the 30 muscles present in each larval abdominal hemisegment we find that only 11 muscles are consistently and specifically activated across segments during rolling. 8 additional muscles are more sparsely activated. Importantly, the sequential pattern of muscle recruitment during rolling is completely distinct from that of forward or reverse crawling. We discover that a roll involves a wave of muscle activation that propagates around the larval circumference (in the transverse plane of each segment) and involves four coactive muscle groups. A pattern of activation progresses from coactive ventral muscle groups to dorsal groups and then spreads across the midline to the contralateral dorsal muscle groups which then progresses back to the ventral groups. We find that the direction of a roll (either clockwise or counterclockwise around the body) is determined by the clockwise or counterclockwise order of muscle group activation around the transverse plane. Finally, based on our knowledge of the order of muscle firing during the rolling behavior, we have determined a putative premotor to motor neuron circuit network from the larval connectome. Results of manipulating the neurons in this circuit, and the affects of these manipulations on the patterned output, will be discussed.

91 **The retrovirus-like genes d*Arc1* and *dArc2* regulate associative learning** Sven Bervoets[1,2], Brennan Dale Mahoney[1], Andrew R Butts[1], Miles Solomon Jacob[1], Jason Dennis Shepherd[2], Sophie Jeanne Cécile Caron[11]School of Biological Sciences, University of Utah, [2]Department of Neurobiology, University of Utah

The activity-regulated cytoskeleton-associated protein (*mArc*) is a key regulator of synaptic plasticity and memory in mammals, while the function of the *Drosophila melanogaster* homologous genes (*dArc1* and *dArc2*) is not fully understood. *mArc* and *dArc* evolved from a ty3-type retrotransposon, but both genes arose independently from two

different repurposing events. The Arc proteins retain some of the ancestral virus-like properties of retrotransposons and still form capsids. Given the high sequence homology, we investigated whether *dArc1* and *dArc2*, like *mArc*, play a role in learning and memory. First, we resolved their expression profiles. We found that *dArc1* and *dArc2* expression levels increase with age. We also found that *dArc1* is expressed in a subset of neurons in the adult fly brain, namely in a small number of serotonergic, cholinergic, and glutamatergic neurons. Second, we performed behavioral studies. We generated flies in which the *dArc* locus, encompassing *dArc1* and *dArc2*, is completely deleted. These *dArc* mutant flies show an intriguing learning phenotype: *dArc* mutant flies form appetitive associations that are stronger than those formed by control flies although both control and mutant flies respond to odor and sugar stimuli just as well. In control flies the *dArc2* expression level, but not that of *dArc1*, increases after learning. We also found that the learning phenotype displayed by *dArc* mutant flies can be rescued by restoring the expression of either *dArc1* or *dArc2* in all neurons. However, when we restore expression with a *dArc1* or a *dArc2* variant that cannot form capsids, we do not observe a rescue of the learning phenotype. Taken together, these data suggest that both dArc proteins are essential for learning in *Drosophila melanogaster* and that this function relies on their ability to form virus-like capsids. Future experiments will determine which neurons release dArc capsids, the functional cargo, and which neurons are the recipient of these capsids. While mammalian and fly Arc were repurposed from separate evolutionary events, our results suggest that their functions have converged as key regulators of learning and memory.

92 **A new dimension to the olfactory system: lncRNAs and a micropeptide upregulated by hunger** Gaelle J. S. Talross, John R CarlsonMCDB, Yale University

Insects rely heavily on their astonishing sense of smell to navigate through our complex environment. The fly olfactory system can detect minuscule amounts of a wide range of odors, and can also finely tune its sensitivity based on the internal state of the animal. Major questions remain about the molecular mechanisms by which the olfactory system changes its sensitivity. Long non-coding RNA (lncRNA) is unexplored in the fly antenna, yet many features of lncRNA could be exploited in olfaction.

We have identified a remarkable diversity of lncRNAs in the fly's main olfactory organ, the antenna, by analyzing bulk and single-nucleus RNA-Seq datasets. We have generated the first lncRNA-to-neuron map of its kind. Our work reveals a new layer of complexity across the olfactory receptor neuron repertoire of the antenna.

We then compared the transcriptomes of starved and fed flies, and identified an ANtenna-enriched RNA that is Upregulated by Starvation by 15-fold, which we renamed *ANRUS*. *ANRUS* is expressed in two different functional types of olfactory sensilla: basiconic sensilla, which sense food odors, and trichoid sensilla, which sense pheromones. ANRUS is classified as a ncRNA and shares many characteristics with other ncRNAs: low GC content, low conservation, and low coding potential. However, to our surprise, *ANRUS* encodes a secreted micropeptide that can be readily detected by antibody staining in the lymph of trichoid sensilla. We are currently exploring the function of the ANRUS micropeptide in trichoid sensilla.

Our work also provides a foundation to explore roles of a wide variety of other lncRNAs in olfactory function and neuronal modulation.

93 **Gliotransmission of D-serine promotes thirst-directed behaviors in Drosophila** Annie Park[1], Vincent Croset[2], Nils Otto[3], Scott Waddell[11]University of Oxford, [2]Durham University, [3]University of Münster

Thirst emerges from a range of cellular changes that ultimately motivate an animal to consume water. Although thirst-responsive neuronal signals have been reported, the full complement of brain responses is unclear. Here, we identify molecular and cellular adaptations in the brain using single-cell sequencing of wa- ter-deprived Drosophila. Water deficiency primarily altered the glial transcriptome. Screening the regulated genes revealed astrocytic expression of the astray-encoded phosphoserine phosphatase to bi-directionally regulate water consumption. Astray synthesizes the gliotransmitter D-serine, and vesicular release from as- trocytes is required for drinking. Moreover, dietary D-serine rescues aay-dependent drinking deficits while facilitating water consumption and expression of water-seeking memory. D-serine action requires binding to neuronal NMDA-type glutamate receptors. Fly astrocytes contribute processes to tripartite synapses, and the proportion of astrocytes that are themselves activated by glutamate increases with water depriva- tion. We propose that thirst elevates astrocytic D-serine release, which awakens quiescent glutamatergic circuits to enhance water procurement.

94 **Central Circadian Clock Control of *Drosophila* Feeding and Activity Rhythms** Sumit Saurabh Saurabh, Ruth

Meier, Liliya Pireva, Daniel CavanaughBiology, Loyola University Chicago

Animals including humans are deeply attuned to their environments and exhibit rhythmic behaviors that follow a 24 hour light-dark cycle. *Drosophila* behavioral rhythms are governed by a set of genes e.g., *period* (*per*) and *timeless* (*tim*) that are present in ~150 of 250,000 neurons in the central nervous system. These neurons constitute a core central clock network that can be functionally and anatomically subdivided into seven different neuronal subsets - dorsal neurons (DN1, 2, and 3), dorsolateral neurons (LNds), small and large ventrolateral neurons (sLNVs and lLNvs) and lateral posterior neurons (LPNs). We asked if different circadian behaviors e.g., feeding and activity, are differentially regulated by these neurons. Using GAL4-inducible gene knockdown via the CRISPR-Cas9 gene editing system, we eliminated *per* and *tim* in selective subsets of clock neurons. We find that free-running feeding rhythms require molecular clock function within multiple individual clock cell populations, and furthermore that the severity of the effect varies according to the cell population targeted. These results parallel those observed when using locomotor activity as a behavioral endpoint, suggesting that circadian control of these two distinct behavioral outputs diverges in downstream circadian output cells rather than in cells of the core clock network

95 **Characterization of tecuzitécatl (*tecu*) mutants in behavioral paradigms** Laura Alejandra Lujano Perez, Juan Rafael Riesgo EscovarUniversidad Nacional Autónoma de México

The brain of *Drosophila melanogaster* (*D. melanogaster*) processes information obtained from different sensory stimuli during its lifetime. Information from various sensory modalities, such as olfaction and vision, flow from the sensory organs to the central nervous system, and are at least partially processed in the brain Mushroom Bodies (MBs). For this study we isolated mutations in a gene we named *tecuzitécatl* (*tecu*), that codes for a secreted phospholipase A_2 (sPA_2) enzyme with a very reduced expression pattern. We show that mutations in this gene lead to faulty behavioral responses in visual paradigms in larvae and adults. We directly compared responses of control (*yw*) and two mutant *tecu* strains: *tecu*[1] and *tecu*[2], both of which have P element insertions within the locus. Both mutants share the same genetic background with the isogenized *yw*control strain. We performed larval phototaxis assays. Results show that mutant strains have significantly different responses (migrate more towards the light), whilst control larvae exhibit negative phototaxis. We then performed adult countercurrent assays, where mutant adult flies also have a defective response to light (mutant flies have significantly less positive phototaxis compared to the control). To elucidate where *tecu*function is needed, we performed electroretinograms on control and mutant flies. Preliminary results from both mutant alleles are consistent with a compromised synaptic communication between the photoreceptors and the laminar interneurons (L1 and L2) of the optic lobes of the brain. Taken together, these results show that *tecu* has an important role processing visual responses in *D. melanogaster*.

96 **Neuronal E93 regulates metabolic homeostasis** Cecilia Yip[1], Steven Wyler[1], Shin Yamazaki[2], Adrian Rothenfluh[3], Syann Lee[2], Young-Jai You[1], Joel Elmquist[21]Internal Medicine, UT Southwestern Medical Center, [2]UT Southwestern Medical Center, [3]University of Utah

Metamorphosis is a transition from growth to reproduction, through which an animal adopts adult behavior and metabolism. Yet, the mechanisms underlying this switch are unclear. Here we report that neuronal *E93*, a transcription factor essential for metamorphosis, regulates the adult metabolism and circadian rhythm in *Drosophila melanogaster.* When *E93* is specifically knocked down in neurons, the flies become hyperphagic and obese with increased energy stores and disrupted circadian rhythms. A screen of Gal4 lines targeting subsets of neurons and endocrine cells identified neurons producing GABA and myoinhibitory peptide (MIP) as the main sites of *E93* action. Repression of the Gal4 driver by Gal80, specifically in MIP neurons, partially yet significantly rescues the *E93* phenotypes, confirming the role of E93 in MIP neurons. Knockdown of the ecdysone receptor specifically in MIP neurons partially phenocopies the MIP neuron-specific knockdown of *E93* suggesting the steroid signal coordinates adult metabolism with E93. Circadian disruption caused by neuronal knockdown of *E93* is also observed when *E93* is knocked down in GABA and MIP neurons. Furthermore, several of the genes enriched in larvae are upregulated in the neuron-specific knockdown of E93. Interestingly, most of them are implicated in neural fate determination (sqz and ko), metabolism (sdr) and circadian rhythm (Clk).

Based on these results, we suggest that neuronal *E93* is a key switch for metabolic transition from the larvae stage to adulthood, representing an intersectional node between metabolism and circadian biology.

97 **Effect of social isolation on gene expression, circuit function and behaviors** Chengcheng Du[1], Qichen C. Duan[2], Jesus C. Sotelo Fonseca[3], Shania C. Appadoo[1], Marta C. Rozados Barreiro[1], Corbin D. Jones[4], Pelin Volkan[51]Biology,

Duke University, [2]Biology, Department of Biology, Duke University, [3]University Program in Genetics and Genomics, Duke University, [4]Biology, UNC-CH, [5]Duke University

Prior social experience modulates variety of animal behaviors. The molecular and circuit-based mechanisms of social experience dependent behavioral modulation remain unclear. Emerging evidence indicates an intimate connection between experience-dependent changes in gene regulation and behavioral modifications. The Drosophila courtship is an excellent model to investigate this question, where links among stereotyped courtship behaviors, genes and circuits have been elucidated. In Drosophila melanogaster, Fruitless^{M} (Fru^{M}) and Doublesex^{M} (Dsx^{M}) are master transcription factors that control sex-specific innate and learned courtship behaviors, respectively. *Fru*^{M} is expressed in 2000 interconnected neurons marking courtship circuits, whereas *dsx*^{M} is expressed in 700 neurons in the nervous system. We and others have found that social isolation increases wild type male courtship vigor whereas monosexual grouping suppresses it. How social experience regulates *fru* and *dsx* expression in the courtship circuits to modify circuit function remains unknown. We found that baseline and evoked activity of neurons within courtship circuits are increased in socially isolated males. In both peripheral and central circuits, social isolation and pheromone receptor mutants regulate chromatin marks around both *fru* and *dsx*, which specifically increase *dsx* but not *fru* expression. These are associated with changes in downstream gene expression programs, altering neuronal sensitivity and courtship behaviors. Blocking different pheromone circuit activity elicited differential effects on gene regulation and particularly pheromone sensing circuits driven by the activity of Or47b neurons contribute to the courtship behavioral differences between grouped and isolated males. These results suggest that social experience and pheromone circuit activity alters the expression of critical genes driving courtship behaviors and neuronal responses within courtship circuits in the brain to ultimately modulate behaviors. Our findings provide insights into the fundamental mechanisms by which sensory experience drive behavioral modulation, via chromatin-mediated changes in the expression of genes critical for neural circuit structure and function.

98 **Small RNA and X-linked repeats collaborate in chromosome identification for dosage compensation** Sudeshna BiswasBiological Science, Wayne State University

Male flies compensate for hemizygosity of their single X chromosome by upregulating X-linked genes. The Male Specific Lethal complex, composed of proteins and *roX* RNA, localizes to the male X and participates in up-regulation. In absence of *roX* RNA, the MSL complex fails to bind the X and male lethality results. *How the MSL complex identifies the X chromosome is poorly understood.* The 1.688^{X} satellite repeats are enriched on the X and contribute to X recognition. Autosomal insertions of 1.688^{X} DNA recruit compensation to nearby genes. Ectopic expression of hairpin RNA (hpRNA) from the pWIZ-1.688^{3F} transgene containing 1.688^{X} sequence from cytological position 3F, produces siRNA, partially restores X-localization of MSL proteins and rescues *roX* mutant males. However, hpRNA from three other 1.688^{X} repeats (1A, 3C, 4A) did not rescue *roX* mutant males. This is baffling as these repeats share 68-95% identity with 1.688^{3F}. My analysis confirmed that all transgenes are intact and express hpRNA. *We hypothesize that details of transgene construction determine function.* To address this, we created a transgene with 1.688^{1A} sequence (89% identity to 1.688^{3F}), but the same orientation and phasing as pWIZ-1.688^{3F} (pWIZ-1.688^{1AR}), and determined that expression of pWIZ-1.688^{1AR} rescued *roX* mutant males. To understand if phasing or orientation was critical, we created pWIZ-1.688$^{1AR\text{-}inv}$, with the phasing of pWIZ-1.688^{1AR} but inverted orientation, and found it also rescued *roX* males. This suggests that phasing, not sequence or orientation, directs hpRNA activity. We then reconstructed transgenes with 1.688^{3C} sequence, sharing 68% identity with 1.688^{3F}, reasoning that if these also support X recognition it is likely that a large number of 1.688^{X} repeats will have the capacity to produce biologically active small RNA. Our findings suggest high redundancy of the siRNAs that contribute to X recognition in male flies. We further speculate that small RNA from these repeats acts to modify chromatin at 1.688^{X} repeats to help identify the X chromosome. Drosophilid X chromosomes are highly enriched for satellite repeats. We propose that repeats facilitate rapid evolution of differentiated sex chromosome by marking X chromatin for compensation.

99 **A novel histone gene array expression and engineering platform with tissue-specific and temporal control for direct interrogation of histone post-translational modification function** Aaron Crain, Markus Nevil, Mary Leatham-Jensen, Katherine Reeves, Greg Matera, Dan McKay, Robert DuronioUniversity of North Carolina at Chapel Hill

Epigenetic regulation of DNA-dependent processes in *Drosophila melanogaster* relies on histone post-translational modifications (PTMs) and the enzymes that modify them. The biological functions of histone PTMs are often interrogated by eliminating the activity of these enzymes, which often have non-histone targets complicating loss-of-function phenotypes. To directly test the function of histone PTMs, we developed a BAC-based transgene platform to rescue homozygous deletion of the ~100 replication-dependent (RD) histone gene units, *HisC*, with a synthetic histone gene

array. Transgenic histone mutant genotypes are generated with each copy of the histone gene of interest containing a PTM-blocking mutation (e.g. K20A). While this approach is powerful it has technical and biological complications. For example, transgenic histone gene arrays are difficult to generate and endogenously expressed histone mutants can cause deleterious phenotypes that impact propagation. Here we present an updated histone gene replacement system with expanded functionality that overcomes many limitations. We used CRISPR gene editing to replace the endogenous *HisC* locus with an engineered locus called *ΔHisC*^{Cadillac} with several key features. First, it precisely deletes *HisC* in contrast to the widely used *Df(2L)HisC*^{ED1429} (*ΔHisC*) that has a 5' deletion of the *lamp1* gene which may negatively impact viability in *ΔHisC* homozygotes. *ΔHisC*^{Cadillac} contains two attP sites for integration of histone transgenes. Notably, histone transgenes integrated into these attP sites can be individually excised using UAS/Gal4-driven B2 and B3 recombinases. The locus also contains *actin5c-dsRed*, simplifying scoring of genotypes with other visible markers and providing a cell marker that is removed upon B2 or B3 excision. This genetically tractable system allows for tissue-specific and temporal control of RD histone expression. We demonstrate integration of histone gene arrays into *ΔHisC*^{Cadillac} and their tissue-specific excision in the anterior compartment of the *Drosophila* wing imaginal disc using *ci-gal4*. To compliment this new, genetically flexible *ΔHisC* locus we engineered a BAC vector to create histone gene arrays via one step Golden Gate Assembly. Additionally, this vector promotes cassette exchange, replacing the *actin5C-dsRed* with an *hsp40-nptii* gene allowing for G418 selection of transformants. Together, this system expands the genetic toolkit of the *Drosophila* chromatin and epigenetics community.

100 **A Tale of Two Condensates: Dynamic interplay between pericentromeric heterochromatin and nucleoli**
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A conserved feature of three-dimensional (3D) nuclear organization is that the nucleolus, the site for ribosome synthesis, is surrounded by pericentromeric heterochromatin (PCH), a condensed chromatin domain essential for chromosome segregation and genome stability. The nucleolus and PCH form membrane-less compartments or condensates that appear via the phase separation of their respective molecular components. However, how these two distinct nuclear condensates assemble proximally and influence each other's organization and functions are unknown. We used high spatial and temporal resolution live imaging during the early nuclear divisions of *Drosophila* development, combined with computational modeling, to determine the *de novo* assembly dynamics of the nucleolus and PCH. We found that nucleolar and PCH condensates are spatially separated during initial nucleation. As they independently grow and self-fuse, nucleoli become tethered to one end of the PCH domain, while the rest of the PCH is in an 'extended' conformation that dynamically transitions between the nuclear lamina and nucleolar periphery. Finally, PCH movements diminish, and it stably surrounds nucleoli. We used mutational analyses of *Drosophila* embryos to investigate whether the organization of nucleoli and PCH is interdependent. Loss of H3K9 methylation, an epigenetic modification enriched at PCH, caused defects in nucleolar size and morphology. Conversely, *Drosophila* embryos lacking ribosomal DNA genes and nucleoli showed dramatic PCH disorganization, characterized by its aberrant clustering at one edge of the nucleus and eventual rearrangement to form a ring-shaped structure. We further discovered that the DEAD-box RNA Helicase, Pitchoune, which normally localizes to the nucleolus, aberrantly accumulated within the PCH ring in embryos lacking rDNA. Together these studies unveil a dynamic program for establishing nucleolar and PCH associations during animal development and demonstrate reciprocal impact on their 3D organization. Further, it suggests that a hierarchy of interaction strengths underlies the 3D nuclear organization of PCH around the nucleolus since disrupting one set of interactions leads others to dominate to create new 3D structures.

101 **Identification of trans-acting factors regulating barrier activity of the Homie chromatin insulator** Savanna F. Lyda[1,2], Catherine E. McManus[1], Juan Manual Caravaca[1], Yang Chen[1], Elissa P. Lei[11]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, [2]Molecular and Cellular Biology, University of Maryland

DNA-protein complexes termed chromatin insulators help maintain genome organization by creating boundaries that separate active and inactive chromatin and controlling promotor-enhancer interactions. 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.

Here, we developed a novel quantitative and tissue-specific *in vivo* reporter of Homie insulator function, demonstrating

that Homie can act as a barrier to the spread of PcG repressive chromatin at an ectopic genomic locus. We found that Homie barrier activity in all tissues tested relies on Rumpelstiltskin (Rump), the hnRNP M homolog in *Drosophila*, as well as two canonical insulator proteins, Centrosomal Protein 190kD (Cp190) and CCCTC-binding factor (CTCF). We next wanted to address whether chromatin compaction at the *eve-TER94* locus may be altered; therefore, we performed 3C analysis in Kc cells after depletion of Rump, Cp190, or CTCF. We found an increase in *cis* looping throughout this region after Rump but not Cp190 or CTCF depletion. We also detected H3K27me3 spreading into the *TER94* locus 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 single Kc cells. This analysis revealed that after Rump depletion, distal PcG domains become closer together in 3D space. However, we did not observe similar changes after either Cp190 or CTCF depletion, suggesting that Rump regulates PcG through a different mechanism than that of the canonical insulator proteins. Taken together, we observe increased compaction of PcG domains in both cis and trans in Rump-depleted cells. Future experiments will address whether Rump-dependent barrier activity at Homie requires Cp190 and/or CTCF.

102 **Heterochromatic 3D genome architecture is directed by H3K9/HP1-dependent and independent mechanisms** Alexis V Stutzman, Christina A Hill, Robin L Armstrong, Riya Gohil, Robert J Duronio, Jill M Dowen, Daniel J McKayUniversity of North Carolina at Chapel Hill

The arrangement of chromatin in three-dimensional (3D) space is important for regulating genome activity and maintaining genome stability. Despite its importance, the molecular mechanisms that control 3D genome organization are largely unknown. A primary correlate of 3D genome organization is chromatin state, raising the possibility that histone post-translational modifications, and the reader proteins that bind them, are key drivers. Here, we focus on the interaction between methylated histone H3 lysine 9 (H3K9) and its reader protein, HP1. H3K9 methylation is strongly enriched at pericentromeric heterochromatin. HP1 binding to methylated H3K9 via its chromodomain is necessary for proper heterochromatin structure and function. Moreover, HP1 is known to multimerize to compact nucleosomes, and it has been proposed to promote self-association of heterochromatin through phase separation. To test the requirement of HP1 and H3K9 in 3D genome organization, we performed *in situ* HiC in *Drosophila* tissues in which the HP1–H3K9 interface has been reciprocally disrupted. To examine the role of HP1, we employed a genotype that harbors a mutation in the HP1 chromodomain that prevents binding to methylated H3K9. To examine the role of H3K9, we engineered flies that express only mutant replication-dependent H3K9 histones that cannot be methylated at K9. We observed strikingly similar phenotypes in each mutant, as well as notable differences. H3K9 methylation and HP1 binding to pericentromeric heterochromatin are lost in both mutants, supporting a requirement of HP1 chromatin binding for proper H3K9 methylation. By contrast, neither mutation impacted HP1 binding to chromosome arms, indicating that HP1 binds euchromatin independently of H3K9 or its chromodomain. HiC reveals significant disruption of pericentromeric boundaries, resulting in increased interactions between heterochromatin and adjacent euchromatin. We also observe an increase in short-range interaction frequency within pericentromeric regions in both mutants. However, despite loss of HP1–H3K9 interactions, we find that self-association of pericentromeric heterochromatin is largely preserved in both HP1 and H3K9 mutants. Together, these observations demonstrate that while HP1–H3K9 interactions contribute to heterochromatin organization, other factors yet to be identified also make major contributions to the segregation of chromatin into euchromatic versus heterochromatic states.

103 **Stonewall links chromatin organization at the nuclear periphery to female germline stem cell fate in *Drosophila*** Ankita Chavan[1], Jailynn Harke[2], Sonny Nguyen[2], Randi Isenhart[2], Noor Kotb[3], Anna Sintsova[4], Prashanth Rangan[5], Eric Joyce[2], Madhav Jagannathan[11]Department of Biology, ETH Zurich, [2]Perelman School of Medicine, University of Pennsylvania, [3]University at Albany SUNY, [4]ETH Zurich, [5]Icahn School of Medicine

The association of chromatin to the nuclear lamina is proposed to facilitate cell-type specific gene repression,leading to distinct cell fates. While identifying proteins that position genomic loci at the nuclear periphery is pivotal to unraveling the link between nuclear architecture and cell fate, these proteins remain relatively unknown in *Drosophila*. In this study, we performed FISH-based high-throughput RNAi screen targeting ~1000 genes to discover novel regulators of nuclear architecture in cultured *Drosophila* cells. We identified *Stonewall* (*Stwl*) as a novel candidate mediating the association of chromatin to the nuclear periphery. Previous studies showed that Stwl is a heterochromatin-associated protein essential for germline stem cell (GSC) maintenance in the *Drosophila* ovary; *Stwl* mutation results in GSC loss, while Stwl overexpression leads to an increase in GSC number. However, the mechanism by which *Stwl* promotes GSC fate is poorly understood. Based on our screen, we hypothesized that Stwl might repress GSC differentiation genes through positioning them at the nuclear periphery. To test this hypothesis, we first identified the Stwl-dependent gene expression profiles

in a GSC-like population. Importantly, our data revealed that Stwl represses known regulators of GSC differentiation, including benign gonial cell neoplasm (bgcn) and meiotic-P26 (mei-P26). Furthermore, we found that the differentiation loci were re-localized from the nuclear periphery to the interior in Stwl depleted GSCs. Strikingly, we also detected gaps in the nuclear lamina in Stwl-depleted GSCs and the lamina gap regions often exhibited impaired chromatin association. Together, our data suggests that Stwl positions genomic loci containing differentiation genes at the nuclear periphery in female GSCs. As a result, Stwl promotes a cell-specific gene expression program ensuring GSC maintenance and female fertility.

104 **Uncovering Novel Functions of Histone Demethylase KDM5 Through a Genome-wide Approach.** Matanel Yheskel, Hayden AM Hatch, Simone Sidoli, Julie SecombeAlbert Einstein College of Medicine

Genetic variants in the human histone lysine demethylase KDM5C gene are associated with the intellectual disability (ID) disorder Claes-Jensen syndrome. KDM5C's main canonical function is the removal of H3K4me3, a histone mark associated with transcriptional activation. Recent studies have shown that KDM5 has both demethylase-dependent and independent functions in neurons. Nevertheless, little is known about the molecular consequences of ID-associated variants in KDM5. Thus, we have modeled patient KDM5C ID-associated variants in *Drosophila melanogaster* to understand how they affect KDM5 promoter recruitment, demethylase activity, transcriptional regulation, and protein-protein interactions. Here, we assay genome-wide KDM5 binding and local changes to H3K4me3, and how these mutations affect transcription of mRNA. We find that while some ID-associated variants affect levels of promoter H3K4me3, this has no effect on gene expression, implicating key roles for non-enzymatic functions of KDM5 in transcriptional regulation. Furthermore, we use the novel technique of proximity labeling to reveal that KDM5 associates with insulator proteins that help partition the genome in 3D-space to ensure proper regulation of genes. KDM5 extensively co-localizes with insulator proteins and topologically associated domain (TAD) boundaries. Interestingly, TAD boundaries are found near housekeeping genes such as ribosomal protein genes (RPGs); a class of gene that is transcriptionally downregulated ID-mutant strains. Importantly, ID variants result in loss of KDM5 binding to insulator proteins. Together, these analyses reveal that changes to interactions with insulator proteins and genome organization may contribute to the cognitive effects observed in individuals with Claes-Jensen syndrome.

105 **Diet-dependent epigenetic silencing of transposable elements** Jennifer McIntyre[1,2], Grace Lee[11]University of California Irvine, [2]Scripps College

Rapidly changing dietary conditions are of concern as to how they influence our genome function and thus health. Transposable elements (TEs) are widespread DNA sequences that are able to replicate and move throughout the genome. TE movement can impact our genome by inducing mutations or altering gene expression, potentially disrupting the function of important genes. Accordingly, it is important to determine how our diet affects this jumping DNA and its potentially harmful consequences. In this project, we aim to investigate how altered diets impact host epigenetic regulation of these selfish genetic elements. To mitigate the replication and movement of TEs, cells deposit repressive epigenetic markers at transposable elements. While this epigenetic silencing successfully limits the movement of TEs, the enriched repressive epigenetic marks can also spread from TEs to neighboring genes. Leveraging this observation, we designed a reporter assay that consists of a reporter gene next to a TE. The altered expression level of the reporter gene informs the extent of TE-mediated silencing of the neighboring reporter gene, which serves as a proxy for the strength of epigenetic silencing of TEs. By rearing flies on various dietary conditions, we discovered that a low-calorie diet leads to enhanced TE silencing, while turmeric and ketogenic diets result in opposite effects. We also tested whether such diet-dependent epigenetic silencing of TEs can be inherited transgenerationally. Intriguingly, while we observed altered TE silencing when both parents and offspring were reared on a low-calorie diet, we failed to find similar diet-dependent effects when either only the parents or the offspring were placed on such diet. Such observations suggest that both transgenerational inheritance and dietary impacts through development are both important. We are conducting whole-genome transcriptomic and epigenomic analyses to further investigate the global impacts of diets on TE silencing. Our study reveals the importance of diet in the regulation of a widespread genomic parasites and could have strong implications for organismal health.

106 **Assessing the connection between obesity and bacterial pathogenesis in a first-year CURE** Moria C ChambersBiology, Bucknell University

Immunity and metabolism are closely intertwined with complex relationships in which alterations to metabolism are beneficial in some infections and not others. For the past two years, students in my first-year Course-based

Undergraduate Research Experience (CURE) at Bucknell University have designed and executed experiments to further develop our understanding of these connections. The course was also designed to promote students' confidence, self-efficacy and interest in biology in their first year with the goal of increasing retention of historically marginalized groups. During the first few weeks, students learned *Drosophila* husbandry and handling, basic microbiological techniques, and how to generate systemic infection in fruit flies. In groups of 3-4, students then developed a hypothesis based on instructor selected readings on bacterial pathogenesis and the metabolic-immune connection. Throughout the rest of the semester students designed and executed three experiments with opportunities to revise and update their hypothesis between each experiment. Students were given access to seven different bacterial species, many different dietary and environmental manipulations and *Drosophila* mutants with a deletion in the adipose gene, which have increased fat storage. Their hypotheses and experiments were diverse, but one highlight was the discovery that the adipose gene had a sexually dimorphic impact on infection with *Providencia rettgeri* infection. Students this year formulated follow-up hypotheses based on the previous years data and are testing whether this sexually dimorphic impact extends to other infections and is affected by rearing the flies on a high sugar diet. Students that participated in the first offering of this CURE reported increased comfort with a wide-range of scientific skills as measured by the CURE Survey with a positive correlation between the gains and instructor emphasis. The framework of this first-year CURE is one that is highly adaptable and could be used for many different questions where gene by environment interactions are important.

107 **Fly-CURE, a Multi-institutional CURE, Has a Positive Impact on Students' Research Self-efficacy, Sense of Belonging in Science, and Interest to Pursue Additional Research Experiences** Julie Merkle[1], Olivier Devergne[2], Paula Croonquist[3], Cory Evans[4], Seth Kelly[5], Kayla Bieser[6], Danielle Hamill[7], Melanie Hwalek[8], Alexandria Pfeister[9], David Puthoff[10], Kenneth Saville[11], Jamie Siders[12], Joyce Stamm[1], Victoria Straub[8], Zully Villanueva[13], Alysia Vrailas-Mortimer[14,15], Jackie Wittke-Thompson[16], Jacob Kagey[171]University of Evansville, [2]Northern Illinois University, [3]Anoka Ramsey Community College, [4]Loyola Marymount University, [5]The College of Wooster, [6]Nevada State College, [7]Ohio Wesleyan University, [8]SPEC Associates, [9]Morehouse College, [10]Frostburg State University, [11]Albion College, [12]Ohio Northern University, [13]Western New Mexico University, [14]Illinois State University, [15]Oregon State University, [16]University of St. Francis, [17]University of Detroit Mercy

The Fly-CURE is a genetics-based multi-institutional Course-Based Undergraduate Research Experience (CURE) that provides undergraduate researchers with hands-on research experiences within a course. Through this CURE, undergraduate students at higher education institutions across the United States (including public, private, community colleges, and minority-serving institutions) map and characterize novel EMS-induced mutants isolated from a Flp/FRT genetic screen in *Drosophila melanogaster*. Undergraduate researchers' successful mapping of over 15 unique EMS mutants has led to local and national scientific presentations by students, as well as nine peer-reviewed publications with over 466 student co-authors. We have also developed and validated assessment tools to study the impact of the Fly-CURE experience on students' self-efficacy and sense of belonging in scientific research, as well as their interest in pursuing additional research experiences. Our data show a gain in these metrics after completion of the Fly-CURE across all student subgroups analyzed, including comparisons of gender, academic status, racial and ethnic groups, and parents' educational background. In addition, we found that students with and without research experience prior to a Fly-CURE course show differential gains in self-efficacy and interest to seek out additional research opportunities. Altogether, our data indicate that the Fly-CURE experience has a significant impact on students' efficacy with research methods, sense of belonging to the scientific community, and interest in pursuing additional research experiences. These data can be used by institutions and departments to develop curricula that provide an optimal number of research opportunities for undergraduate students.

108 **An advanced Genomics Education Partnership CURE exploits long-read genome assemblies to study F Element expansion in four *Drosophila* species** Timothy J Stanek[1,2], Wilson Leung[3], Nicole S Torosin[1], Christopher D Shaffer[3], Cindy J Arrigo[4], Christopher E Ellison[11]Genetics, Rutgers University, [2]Pathology and Laboratory Medicine, Robert Wood Johnson Medical School, [3]Biology, Washington University in St. Louis, [4]Biology, New Jersey City University

The Muller F element is the smallest chromosome (~5.2 Mb) in the *Drosophila melanogaster* genome. Despite being almost entirely heterochromatic, the banded portion of the chromosome (~1.3 Mb) contains ~80 protein-coding genes with expression levels similar to genes in euchromatic domains. Past studies have shown that the F elements of several *Drosophila* species are larger than the *D. melanogaster* F element. To identify the major contributors to F element expansion and to assess the impact of this expansion on F element genes, we performed a study of four *Drosophila* species whose F elements have undergone various levels of expansion. We constructed genome assemblies for *D.*

kikkawai, *D. takahashii*, and *D. bipectinata* using PacBio, Nanopore, and Illumina sequencing data. Hi-C data was then used to determine the relative order and orientations of the scaffolds in these three genome assemblies and in the *D. ananassae* PacBio assembly produced by The University of Maryland. These chromosome-level assemblies show that the region spanning the F element genes has undergone 2- to 16-fold expansion compared to *D. melanogaster* (from ~2.2 Mb in *D. kikkawai* to ~19.8 Mb in *D. bipectinata*). During the past three years, Genomics Education Partnership (GEP) students have engaged in structural gene annotations of *D. ananassae*, *D. bipectinata*, and *D. kikkawai* in local CUREs. In Fall 2022, we developed an online, multi-institutional CURE that engages students in the final reconciliation of genes on the *D. takahashii* F element and on a euchromatic region of the Muller D element, resulting in updated, high-quality gene annotations for these regions. Students have also investigated other interesting features in these domains, including strain differences, assembly and sequencing errors, novel or lost isoforms, and pseudogenes. This online CURE is available as an independent research course to students with prior F Element GEP annotation experience, with a designated faculty mentor at their institution. Led by a Rutgers IRACDA postdoctoral scholar, the course consists of independent research guided by weekly meetings with other course participants, peer mentors, and GEP faculty to ensure standardized practices and a supportive, safe, and inclusive environment. For more information, contact us at thegep.org/contact. Supported by NSF grants 2114661 and 1915544 and NIH grants K12GM093854 and R25GM1305.

109 **A framework for educating and empowering students by teaching about history and consequences of bias in STEM** Andrea M Darby[1], Amelia-Juliette C Demery[2], Lina M Arcila Hernández[2], Clara L Meaders[3], Corrie S Moreau[11]Entomology, Cornell University, [2]Ecology and Evolutionary Biology, Cornell University, [3]Division of Biological Sciences, Section of Cell and Developmental Biology, University of California, San Diego

Racism and bias are pervasive in society-and science, technology, engineering, and mathematics (STEM) fields are not immune to these issues. It is imperative that we educate ourselves and our students about the history and consequences of this bias in STEM, investigate the research showing bias toward marginalized groups, understand how to interpret misuses of science in perpetuating bias, and identify advances and solutions to overcome racism and bias throughout our professional and personal lives. Here, we present one model for teaching a universal course for participants of all professional stages to address these issues and initiate solutions. As very few institutions require students to enroll in courses on racism and bias in STEM or even offer such courses, our curriculum could be used as a blueprint for implementation across institutions. Ultimately, institutions and academic disciplines can incorporate this important material with more region and/or discipline specific studies of bias.

Integrating culture and community in diabetes research, an intro bio collaboration Elyse Bolterstein, Sarah Alfaqih, Fatima Alguzi, Dahlia LouNortheastern Illinois University

It is well-accepted that hands-on classroom approaches such as Inquiry-based learning are effective in training the next generation of scientists. However some students, especially those from groups underrepresented in the sciences, may not yet feel valued in the scientific community. Culturally relevant pedagogy can increase inclusion in the sciences by acknowledging that students' unique backgrounds and experiences enhance scientific progress. These practices are of critical importance in retaining biology majors at our institute, which is classified as a Hispanic Serving Institute and the most diverse public comprehensive university in the Midwest. Therefore, we adopted inquiry-based projects and culturally relevant practices in revising our introductory course for biology majors. Our goal was to increase inclusion and build community within our majors while teaching basic research and laboratory skills. Through the central class theme of diabetes, students explored health disparities, evaluated data mapping diabetes incidence to various social and economic factors, and performed original research connecting diabetes and nutrition. The research component was in collaboration with eCLOSE, a non-profit organization dedicated to supporting educators and students in research that directly contributes to research questions connecting nutrition and disease. Our students learned about pathways connecting the gene Pi3K with cellular glucose uptake and then treated wildtype and Pi3K mutant Drosophila with various dietary nutrients chosen for their relevance to different cultural communities. Students collected and analyzed data showing how the dietary nutrients influenced offspring number and development. Students presented their findings in a class poster session that was shared with the eCLOSE community. At the end of the semester, students reported strong project ownership and inclusion in the scientific community

110 ***Drosophila melanogaster* isofemale line models in undergraduate Genetics laboratories: Genetic architecture of natural populations** Guy F Barbato[1], Anrew Walsh[2], Colleen Dougan[11]Biology, Stockton University, [2]Stockton University

Many models exist to measure the relative proportion of both genetic and environmental variation for phenotypes of any type. However, most models use pre-established populations, denying students the opportunity to build their own model and to directly estimate populational variation. Generally, these models - whether Mendelian or molecular - give the impression that phenotypic variation of fitness traits is discontinuous and/or qualitative. An isofemale strain design allows for the persistence of line differences across many generations, as determined by a single, wild-caught female, and allows for phenotypic screening of nearly endless phenotypes that catch a students interest.

In this case, students in my Genetics labs over the last 2 years have collected 41 samples of wild *Drosophila melanogaster* from across the state of New Jersey and founded three (3) replicate isofemale lines from each collection. Students are involved in every phase of population maintenance and breeding; learning husbandry and genetic techniques. We currently have 115 lines, 85 of which have been maintained for over 30 generations. Twelve students have been working on the research project since its inception.

Surprisingly, inbreeding did not decrease fecundity among the lines, but populations produced consistent numbers of progeny throughout their maintenance. We further observed that pupal counts at 7d post-mating varied by two orders of magnitude among the lines.

Students have begun collecting data on testis morphology and sperm production, egg laying and responses of the lines to extremes of temperature and dietary changes. Genetic variation has been observed for all phenotypic categories and data have been presented at regional undergraduate research symposia. We have also collected and isolate genomic DNA from all lines for future analysis.

111 **Decoding Transcription Factors: A semester long bioinformatics CURE identifying DNA-binding factors at a specific genetic locus** Lauren Hodkinson, Casey Schmidt, Skye Comstra, Leila RiederDepartment of Biology, Emory University

Implementing Course-based Undergraduate Research Experiences (CUREs) can allow for accessible, authentic research opportunities for students who might be limited by available space, time, and laboratory resources. CUREs can even be curated to address challenges, such as the COVID-19 pandemic, that further limit student access to research. Recently, our lab developed and piloted a bioinformatics CURE with students who were restricted from physically working in laboratories due to the pandemic. Our lab is interested in the regulation of the *Drosophila* histone genes, and our goal was to screen for candidate histone gene regulators using publicly available high-throughput datasets. This past spring, we implemented the CURE in an introductory genetics course of 25 students that included a 50-minute weekly discussion period for the project. First, students used available databases and publications to form hypotheses about candidate DNA-binding factors related to histone gene regulation. Next, the students found existing ChIP-seq datasets from NCBI GEO. The students then mapped reads to the *Drosophila* histone gene array using Galaxy, a free web-based platform that integrates bioinformatics tools and makes them usable for individuals with no coding experience. Students visualized their data using the open-source Integrative Genomics Viewer (IGV) software. Finally, students created a research poster and presented their findings in a formal session to an audience of their peers as well as invited faculty. Overall, the students studied 27 different factors and we identified 9 promising candidates that future students will investigate in our wet lab. Our CURE introduced students to hypothesis-driven research, gave them experience with common bioinformatic analyses, and allowed them to participate in research in a more flexible, accessible environment. Beyond the scope of our CURE project, the students gained skills to map any ChIP-seq dataset to any genome or specific locus. Furthermore, bioinformatics can be performed outside of the traditional laboratory setting and can be applied to a wide array of different scientific questions, making it a more inclusive opportunity to increase undergraduate participation in scientific research.

112 **Freshman Research Initiative Behavioral Neuroscience course for undergraduate research training in neuroscience** Thilini Wijesekera, Nigel AtkinsonNeuroscience, University of Texas at Austin

The Freshman Research Initiative (FRI) of the University of Texas at Austin is an undergraduate research program comprised of 35 research education streams spanning various disciplines of science. This program is one of the first and largest of its kind in the country, and has been in existence for nearly 20 years. The program's goal is to provide authentic research experiences to freshman undergraduates based on their scientific interests. Each course spans a period of two semesters of which the initial Spring semester is mostly pedagogical, where freshmen are educated on scientific method and conducting a research study. The Fall semester is focused on independent research. The Behavioral Neuroscience stream is a relatively new addition to the FRI. The stream was initiated with the goal of

providing an FRI experience specialized in neuroscience research, to students inclined towards neuroscience. The stream uses *Drosophila melanogaster* as a model organism and investigates questions pertaining to the functioning of neural circuits and genes in the context of behavior. The Spring semester curriculum provides a thorough knowledge of the rich genetic potential of the system as well as hands-on experience on designing and conducting fly behavior experiments. It also trains students in scoring and statistical analysis of behavioral data, and interpretation of findings as well as critical reading of scientific literature and formulating research questions and experimental design. The Fall semester focuses on independent research studies based on the concepts and techniques learned during the Spring semester, while accommodating a few specialized topics. Additionally, it carries a writing flag, which develops scientific writing in students, also in the context of fly neuroscience. **This stream employs cost-effective approaches to expand research material to accommodate classroom-based research. We present economical ways to collect and store fly reagents and equipment for behavior experiments, such as courtship, associative conditioning, alcohol sensitivity, and optogenetics. These are highly adaptable to novice undergraduate researchers and classroom conditions.** Collectively, the FRI Behavioral Neuroscience stream is a research education program that caters to the neuroscience interests of undergraduate students and beyond.

113 **Paths and pathways that generate cell-type heterogeneity and developmental progression in hematopoiesis** Juliet R Girard[1], Lauren M Goins[2], Dung Vuu[2], Utpal Banerjee[21]Biology, University of Massachusetts Boston, [2]University of California Los Angeles

Insights into the signaling pathways and developmental transitions that underlie hematopoiesis can illuminate the underlying causes of blood diseases such as leukemia. The blood system of Drosophila melanogaster represents a genetically-tractable model to understand human hematopoiesis. Study of hematopoiesis in Drosophila has been limited by the use of a small number of markers that are shared between several cell types. We used transcriptomic dissection of hematopoiesis to address this problem, allowing us to resolve the heterogeneity of cell types and to determine the developmental progression between these subpopulations. We profiled the global gene expression in the blood-forming organ (lymph gland) using a combination of bulk RNA sequencing (RNA-Seq) on FACS-separated populations and single cell RNA-Seq. These analyses reveal several new blood cell subpopulations that were mapped along a developmental trajectory going from an early progenitor state to individual mature differentiated cell types. We used these data as a framework to generate hypotheses that could then be tested with in-depth genetic analysis. For example, we find two distinct subpopulations that represent intermediate states of differentiation, which define two major paths of development. These intermediate cell types engage in different modes of signaling with neighboring cells, both of which play important roles in hematopoiesis. We also observed that a mature cell population known as crystal cells had two distinct subpopulations and uncovered a mechanism involved in the transition between them. We found that during this transition, crystal cells switch between ligand-dependent and ligand-independent Hif-dependent Notch signaling. This switch requires Numb upregulation in mature crystal cells, which promotes Notch/Hif signaling and crystal cell maturation. Numb levels are kept low in immature crystal cells by the action of the RNA binding protein Musashi (Msi), but rise when Msi levels plummet in mature crystal cells. We demonstrate the rise of a specific splice variant of Hif that is Tango-independent and present only in mature crystal cells. The findings garnered through the combination of RNA-Seq with detailed genetic dissection outlined here may provide new understanding of the signaling pathways involved in mammalian hematopoiesis.

114 **Cytonemes coordinate asymmetric signaling and organization in the *Drosophila* adult muscle progenitor niche** Akshay Patel[1], Yicong Wu[2], Xiaofei Han[2], Yijun Su[2], Tim Maugel[3], Hari Shroff[2], Sougata Roy[41]Cell Biology and Molecular Genetics, University of Maryland, [2]Laboratory of High-Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, [3]Biology, Laboratory for Biological Ultrastructure, University of Maryland, [4]University of Maryland, College Park

Tissue development and homeostasis rely on the ability of stem cells to efficiently balance between self-renewal and differentiation. These fate decisions are made in the context of the niche and are governed by asymmetric signaling and organization within the niche. Niche cells present self-renewal growth factors selectively to stem cells, but not to their neighboring non-stem cell daughters, often located just one cell diameter away. Understanding how this signaling asymmetry arises is critical to understand how stem cells determine their identity and prime differentiation in an organized pattern to generate/regenerate tissues. By employing genetic and cell-biological techniques, along with high-resolution microscopy, we investigated the basis of asymmetric signaling and cell organization in the *Drosophila* wing disc that creates an Adult Muscle Progenitor (AMP) niche. We showed that AMPs extend polarized actin-based filopodia, called cytonemes, to directly contact the wing-disc epithelial junctions and physically adhere AMPs to the wing disc

niche. This cytoneme-mediated niche adhesion helps AMPs to maintain their niche occupancy, polarity, and stemness. We further investigated how cytonemes polarize and selectively adhere to the wing disc niche. We found that AMP cytonemes localize an FGF-receptor, called Heartless (*htl*), to selectively adhere only to FGF-producing wing-disc cells and directly receive FGFs in a contact-dependent manner. This target-specific FGF reception and signaling activation only in the adhering AMPs, in turn, determines the niche-specific polarity and adhesion of AMP cytonemes. Loss of cytoneme-mediated adhesion promotes AMPs to lose niche occupancy and FGF-signaling and acquire morphological hallmarks of differentiation. Furthermore, we found that the wing disc expresses two Htl ligands, Pyramus (*pyr*) and Thisbe (*ths*), in non-overlapping domains. Pyr and Ths signal to cells by binding to the common Htl receptor, but contact-mediated delivery of each ligand via cytonemes induces AMPs to acquire distinct direct- and indirect- flight muscle-specific fates in spatially distinct niches. These findings provide new insights into how restricted expression/presentation and polarized contact-dependent exchange of FGF via cytonemes can generate and maintain diverse niche-specific asymmetric signaling and organizations of stem cells.

115 **Nuclear Actin is a Critical Regulator of Drosophila Germline Stem Cell Maintenance** Nicole M Green, Danielle Talbot, Tina L TootleAnatomy & Cell Biology, University of Iowa

While actin was observed in the nucleus decades ago, nuclear functions of actin have only recently been widely acknowledged. Nuclear actin regulates the activity of RNA polymerases and transcription factors, chromatin organization via remodeling complexes and histone deacetylases, and nuclear integrity. Furthermore, nuclear actin has been implicated in regulating differentiation, deciding cell identity, and reprogramming cells to a pluripotent state. However, its roles in development and tissue homeostasis remain largely unknown. Here we uncover the roles of nuclear actin in regulating stemness using a model tissue, the *Drosophila* ovary. The *Drosophila* ovary is made up of 15-20 ovarioles of sequentially developing follicles. Germline stem cells (GSCs) give rise to all germline cells and reside in a niche at the anterior tip of each ovariole in a structure known as the germarium. We found that nuclear actin is dynamic in the early germ cells. Specifically, nuclear actin recognized by anti-actin C4 is found in both the nucleoplasm and nucleolus of GSCs. The nucleoplasmic C4 pool, which is polymeric nuclear actin, is lost at the 2-cell stage, whereas the monomeric nucleolar pool persists to the 8-cell stage. This finding led us to hypothesize that polymeric nuclear actin plays a critical role in regulating stemness. To test this hypothesis, we over-expressed NLS-actin constructs to alter nuclear actin polymerization states in the GSCs and their daughters. Strikingly, increased monomeric nuclear actin (NLS-Act5C^{G13R}), but not WT actin (NLS-Act5C) which can polymerize, results in GSC loss that ultimately causes a progressive loss of the germline loss. This GSC and germline loss is rescued by simultaneous over-expression of monomeric nuclear actin and WT nuclear actin. Together these data reveal that GSCs require polymeric nuclear actin. This polymeric nuclear actin likely plays numerous roles in the GSCs, as increasing monomeric nuclear actin (NLS-Act5C^{G13R}) disrupts nuclear architecture causing nucleolar hypertrophy, distortion of the nuclear lamina, and heterochromatin reorganization; all factors critical for GSC maintenance and function. These data provide the first evidence that nuclear actin, and in particular its ability to polymerize, are critical for stem cell function and tissue homeostasis.

116 **The conserved RNA binding protein Orb2 regulates cell-type-specific responses to rare codon enriched transcripts during neural stem cell differentiation** Rebeccah Stewart[1], Scott Allen[2], Don Fox[11]Duke University, [2]UNC Chapel Hill

Differences in gene and 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 and 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 a single stem cell lineage. Neural stem cells are unable to express reporters or endogenous genes enriched in rare codons, while differentiated neurons do so robustly. Differentiation is tied to the ability to express transcripts enriched in rare codons, as experimentally blocking neuronal differentiation drastically reduces rare-codon enriched reporter protein. Artificially increasing abundance of rare-codon enriched transcripts leads to cell death specifically in neural stem cells. 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 expression in the brain. 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. 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 a defined stem cell lineage that drives cellular differentiation and function.

117 **Blastema transcriptional state is sustained by transcription factor Ets21C during imaginal disc regeneration and tumorigenesis** Melanie I. Worley[1], Nicholas J Everetts[2], Riku Yasutomi[2], Rebecca J Chang[2], Nir Yosef[2], Iswar K Hariharan[21]Biology, University of Virginia, [2]UC Berkeley

Regeneration requires surviving cells to mount a response that promotes localized proliferation and repatterning to replace lost and damaged tissue. In *Drosophila*, the larval imaginal discs regenerate through the formation of a blastema, a zone of localized cell proliferation and increased cellular plasticity. Many important processes during regeneration occur in small subpopulations of cells, the study of which has been revolutionized by single-cell technologies. By profiling the transcriptomes of thousands of individual cells from developing and regenerating imaginal discs, we have identified regeneration-specific transcriptional programs and unique cellular states, including two distinct cell populations within the blastema. These regeneration-specific cell states are characterized by the upregulation of a myriad of genes encoding secreted proteins that establish the pro-regenerative microenvironment. The transcription factor Ets21C is specifically expressed during regeneration in this regenerative secretory zone, and we have demonstrated that Ets21C controls the expression of multiple regeneration-promoting genes, including *Mmp1*, *Ilp8*, *upd3,* and *asperous*. While eliminating Ets21C function has no discernible effect on development, it severely compromises regeneration. Regenerating tissues in *Ets21C*$^{-/-}$ mutants fail to maintain a less differentiated blastema and to pause tissue-wide transcriptional changes. As a result, regenerative growth terminates prematurely. Thus, the Ets21C-controlled transcriptional program within the blastema cells is required to effectively coordinate a regenerative response. We also find that this Ets21C-dependent gene regulatory network is activated in small populations of blastema-like cells in tumorous discs, suggesting that pro-regenerative mechanisms can be co-opted by tumors to promote aberrant growth. Our findings highlight unappreciated heterogeneity within the imaginal disc blastema, reveal a critical regenerative gene regulatory network orchestrated by Ets21C, and suggest that this gene regulatory network might function in subpopulations of cells to organize both regenerative and tumorous growth.

118 **Epithelial Ca^{2+} waves triggered by enteric neurons heal the gut** Afroditi PetsakouHarvard Medical School

An unresolved question in regenerative biology is how tissues transition to homeostasis after injury. Answering this question is vital for unmasking the etiology of various chronic disorders like inflammatory bowel diseases and cancer. We addressed this using the *Drosophila* gut, a powerful regenerative model and made the striking observation that recovery after injury depends on the highly conserved cholinergic pathway. Specifically, reduction of nAchRs (nicotinic Acetylcholine Receptors) in ECs (enterocytes, the main cell type of the *Drosophila* gut) after injury maintains the intestinal epithelium in an over-inflammatory and over-proliferative state rendering it unable to recover, whereas increase of nAchRs in ECs expedited recovery restoring the epithelium to a state identical to controls. In addition, we identified a small population of cholinergic enteric neurons, referred as ARCENs (Anti-inflammatory Recovery-specific Cholinergic Enteric Neurons), that are required for nAchRs in ECs to advance gut recovery. Further, we found that activation of nAchRs in ECs, that are innervated by ARCENs, initiates Ca^{2+} currents that spread across the tissue by epithelial gap junctions promoting ion balance, epithelial maturation and reducing inflammation. Altogether, we discovered that cholinergic neuro-epithelial communication triggers epithelial gap junctions to spread a Ca^{2+} wave that heals the gut after damage.

119 ***Brainwashing* regulates sphingolipid and fatty acid saturation to promote intestinal stem cell proliferation in *Drosophila* midgut** Mahi Rahman[1], Chloe Kraft[2], Collin Clark[2], Marco Marchetti[3], Chenge Zhang[2], William Holland[4], Scott A. Summers[4,5], Bruce A. Edgar[21]Oncological Sciences, Huntsman Cancer Institute, [2]Oncological Sciences, Huntsman Cancer Institute, Utah, [3]3I-UCGD Bioinformatics Core, [4]Nutrition & Integrative Physiology, University of Utah, [5]Diabetes and Metabolic Research Center

High-fat diets and fatty acid oxidation regulate stem cell proliferation. Sphingolipids such as ceramides are a major component of high-fat diets. However, the molecular mechanisms involved in ceramide metabolism-mediated intestinal

stem cell (ISC) proliferation and tumorigenesis are poorly understood. A predicted alkaline ceramidase *brainwashing* (Bwa) shares a significant amino acid homology with mammalian alkaline ceramidase Acer2. In *Drosophila*, however, Bwa has been shown to have no ceramidase activity, and the nature of its enzymatic activity is unknown. Bwa genetically interacts with the other predicted *Drosophila* ceramidase (*cdase*) and with ceramide kinase (*cerk*), and can regulate sphingolipid flux. To understand how Bwa-mediated changes in the sphingolipid metabolite flux affect intestinal stem cells, we manipulated the *bwa* levels using cell type-specific Gal4 drivers in the adult midguts. Bwa over-expression in the ISCs promoted ISC proliferation and increased ISC size. Enterocyte (EC) and enteroendocrine (EE)-specific *bwa* over-expression did not alter the morphology of the ECs or EEs, although it increased cell non-autonomous ISC proliferation.

Interestingly, bwa over-expression in gut enteroblasts (EB) increased EB cell size and caused a non-cell-autonomous 7-8 fold increase in ISC proliferation. Bwa knockdown too, increased proliferation but only by 1.5 -2 fold compared to control cohorts. Further characterization showed that bwa over-expression is associated with higher Notch activity and an increase in all the cell types, suggesting that Bwa gain of function increased ISC proliferation without affecting the differentiation potential of the EBs. Targeted and untargeted lipidomic analysis by mass spectrometry showed that *bwa* overexpression caused a robust accumulation of both saturated sphingolipid metabolites and saturated fatty acids. In addition, we found that co-expression of the gene infertile crescent (*ifc*), which desaturates dihydroceramide by introducing a carbon chain double bond, counteracted the pro-proliferative effect of bwa overexpression. Together, these results suggest that high levels of saturated sphingolipids, and possibly other saturated fats, promote ISC proliferation. Pseudo-epistasis analysis showed that the accumulation of saturated sphingolipids and fatty acids induces inflammatory signaling and activates ISC proliferation through the Upd3/JAK/STAT pathway. We propose that Bwa might be a regulatory protein that inhibits desaturase enzymes and thereby causes the accumulation of saturated sphingolipids and fatty acids.

120 **Identifying factors that maintain a stem cell niche** Gabriela S Vida, Elizabeth Botto, Vaibhav Desikan, Stephen DiNardoUniversity 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, 2021), and begun illuminating the cell mechanics involved in first forming a compact, functional niche (B. Warder). 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 maintain a balance of renewing GSCs and differentiating cells. Our preliminary evidence suggests that acto-myosin contractility (AMC) is important for the maintenance of the spherical nature of the stem cell niche. Furthermore, abolishment of this spherical structure reduces the effectivity of niche signaling per GSC, and instead allows for more germ cells to respond to signaling. Finally, this loss of niche structure causes the GSCs to misorient their centrosomes, potentially disrupting the GSC division program. Ultimately, this work shows a novel way in which niche structure ensures a proper balance of stem cells and is crucial for their behavior.

121 **Evolutionary diversification of Arp2 separated somatic versus germline roles** Kaitlin A Stromberg[1], Tristan Spain[1], Sarah A Tomlin[2,3], Courtney M Schroeder[41]UT Southwestern Medical Center, [2]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, [3]University of Washington, [4]Pharmacology, UT Southwestern Medical Center

The actin cytoskeleton often forms branched networks that are critical in many fundamental cellular processes, including cell motility and division. Arp2, a protein within the 7-membered Arp2/3 complex, is responsible for generating branched actin and is under stringent sequence conservation throughout eukaryotic evolution. However, we unexpectedly discovered diversification of Arp2 in two clades of *Drosophila* species: gene duplicates *Arp2D* in the *obscura* clade and *Arp2D2* in the *montium* clade. Our targeted sequencing and phylogenetic analyses show these duplicates evolved independently 14 million years ago and were retained throughout speciation within their respective clades. The two duplicates exhibit distinct sequence diversification from canonical Arp2 and are unusually testis-enriched in expression in their native species. We investigated whether these gene duplicates' sequence divergence has led to diversification of canonical Arp2's function. We replaced canonical *Arp2* in *D. melanogaster* with *Arp2D* or *Arp2D2* to test whether both duplicates can rescue the *Arp2*-KO lethality phenotype. Interestingly, despite their divergence, we found both rescue lethality, suggesting they can polymerize branched actin like canonical Arp2. However, *Arp2D*-expressing males were

subfertile. Cytological analyses confirmed Arp2D can generate branched actin yet revealed many defects throughout sperm development. We hypothesized that Arp2D's unusually long and charged C-terminus, which is absent in canonical Arp2 and Arp2D2, might lead to the observed fertility defects. We removed Arp2D's C-terminus, and surprisingly, it could no longer rescue lethality despite being more structurally similar to canonical Arp2. We then tested the function of canonical Arp2 with Arp2D's C-terminus ("Arp2-Ct") and found that Arp2-Ct-expressing flies are viable yet exhibit testis defects. Therefore, while the divergent Arp2D's novel C-terminus is essential for its somatic function, this divergence is incompatible with *D. melanogaster* sperm development. Overall, our findings suggest canonical Arp2's roles can be partitioned into somatic versus germline roles, and testis-specific Arp2 has recurrently evolved for specialized actin branching.

122 **The role of microtubule motor adaptor proteins in controlling mitochondrial movement during oogenesis** Matthew J Gillen[1,2], Rachel Cox[11]Biochemistry, Uniformed Services University, [2]Biochemistry, Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

The Milton/Miro cargo adaptor complex that links mitochondria to the motor protein kinesin and dynein. Miro is an atypical GTPase with a transmembrane domain that inserts in the mitochondrial outer membrane. Milton is an adaptor protein that binds to Miro and Kinesin. In Drosophila, there are several isoforms of Milton. This cargo complex then moves mitochondria along the microtubules. The Drosophila ovary is an excellent place to study mitochondrial movement along microtubules because the microtubule polarity is well-established in the germ cells and the surrounding follicle cells. In addition, germ cells form interconnected cysts in many species, from Drosophila to humans. Since mitochondria are exclusively maternally inherited, mitochondrial transport between germ cells could affect inheritance. Our previous work showed that altering levels of two Milton isoforms affected mitochondrial movement towards the plus- and minus-ends of microtubules. Class II *milton* alleles caused uncontrolled mitochondrial movement into the oocyte towards the microtubule organizing center. This suggested that Milton not only directly binds kinesin but can affect dynein-based movement as well. In agreement with this hypothesis, recent studies of the vertebrate Milton homolog, TRAK1 and 2, have shown TRAK1 binds Kinesin and Dynein/Dynactin to control mitochondrial movement. The primary goal of this study is to further understand the basic mechanism of how Milton and Miro influence mitochondrial movement along microtubules during oogenesis. We are further characterizing the additional Milton isoforms in order to determine how they affect mitochondrial directional movement, whether they differentially bind Kinesin and Dynein/Dynactin and whether there are tissue-specific effects of isoform-specific knockdown. We will present evidence that altering these additional isoforms affects plus- and minus-end directed mitochondrial movement into the oocyte and in the surrounding follicle cells. Since a subset of mitochondria is deposited into the Drosophila oocyte during oogenesis, controlling movement could be one way to select healthy vs unhealthy mitochondria for the next generation. In addition to deepening our basic knowledge of molecular motor control of mitochondrial transport, this study could help identify part of the mitochondrial quality control system governing mitochondrial inheritance. This could have implications for maternally inherited forms of mitochondrial disease.

123 **Sac1, a PI4P phosphatase, maintains epithelial integrity during Drosophila dorsal closure** Kimberley D. Gauthier[1], Julie A. Brill[1,21]Cell Biology, The Hospital For Sick Hospital, [2]Department of Molecular Genetics, University of Toronto

The development and repair of epithelial tissue relies on epithelial sealing, involving the formation of new cell junctions for a seamless seal. Dysregulation can lead to developmental anomalies such as cleft palates and neural tube defects. Epithelial sealing occurs during Drosophila embryogenesis in a process called dorsal closure, in which the epidermis wraps around the developing embryo, covering an extraembryonic epithelium (the amnioserosa) and zippering shut along the dorsal midline. Dorsal closure is driven by cell constrictions in the amnioserosa, in addition to JNK MAPK signaling and formation of an actomyosin cable in the leading edge of the epidermis. Previously, the Brill and Harden labs found that embryos with mutations in *Sac1*, an ER-associated PI4P phosphatase, died during embryogenesis and failed to complete dorsal closure, concomitant with ectopic JNK signaling and irregular amnioserosa cell constrictions. How Sac1 and PI4P homeostasis regulate cellular mechanics driving dorsal closure remains unknown. Using time-lapse confocal microscopy to image fluorescent markers for non-muscle myosin and adherens junctions, I found that *Sac1* mutants have cell-cell adhesion defects in the amnioserosa, ranging from loss of E-cadherin after cell delamination to tissue tearing, paired with formation of an actomyosin cable. In addition, *Sac1* mutants progress through dorsal closure slower than controls and exhibit epidermal puckering characteristic of JNK overactivation. Despite these defects, a majority of *Sac1* mutants successfully completes dorsal closure. Puckering and epithelial tearing are suppressed by co-expression of a variant of myosin heavy chain, consistent with a genetic interaction between Sac1 and myosin. Surprisingly, there were

no detectable changes in PI4P levels in *Sac1* mutants; instead, $PI(4,5)P_2$ levels were slightly increased in amnioserosa cell membranes. Finally, *Sac1* mutants have reduced fluorescence of the myosin-activating Rho kinase and modestly reduced levels of phosphorylated myosin at cell junctions in the amnioserosa, pointing to dysregulation of myosin activity. These results suggest that Sac1 maintains amnioserosa integrity and epidermal morphogenesis via a myosin-dependent pathway. Future experiments will uncover the molecular mechanisms through which Sac1 and phospholipids keep epithelia intact. This research will further our understanding of how phospholipid homeostasis orchestrates cellular forces that drive development.

124 **Phase separation properties determine the *in vivo* function of adaptor proteins in myoblast fusion** Zhi-Rong Ruan, Ruihui Zhang, Danqing Tong, Elizabeth ChenMolecular Biology, UTSouthwestern Medical Center

Cell surface transmembrane receptors often form nanometer- to micrometer-scale clusters to initiate signal transduction in response to environmental cues. At the sites of *Drosophila* myoblast fusion known as the fusogenic synapse, Ig domain-containing cell adhesion molecules (CAMs) accumulate in clusters and recruit over a dozen additional proteins to facilitate cell membrane fusion. These proteins are enriched in a “focus” to promote actin polymerization and invasive membrane protrusions. The SH2 and SH3 domain-containing adaptor proteins, Crk, Drk, and Dock, are known to link CAMs and the actin cytoskeleton. However, which adaptor protein replays the cell fusion signal from the CAM, Sticks and stones (Sns), to the actin cytoskeleton remains a longstanding question. Using an *in vitro* phase separation assay, we found that the cytodomain of Sns phase separated by itself and that Crk, but not Drk or Dock, partitioned into the Sns condensates. Correspondingly, Crk was recruited to the liquid droplets formed by Sns cytodomain in cultured S2R+ cells. Moreover, *crk*, but not *drk* and *dock*, mutant embryos exhibited a severe myoblast fusion defect, despite the accumulation of Sns and F-actin at the fusogenic synapse. EM analysis showed that in *crk* mutant, the invasive protrusions were abnormally wider, indicating disorganized actin filaments within these protrusions. We further show that the actin-binding WASP-interacting protein Solitary (Sltr) was no longer recruited to the fusogenic synapse in *crk* mutant embryos. Taken together, our study demonstrates that the distinct phase separation properties of the small adaptor proteins determine their *in vivo* function in myoblast fusion, and suggests that Sltr is recruited to the Sns-Crk condensate at the fusogenic synapse to increase the mechanical strength of the actin cytoskeleton, allowing the formation of narrow invasive protrusions to promote cell membrane fusion.

125 **Repressing a Repressor: How Germ Cells Form Despite Somatic Interference** Mariyah Saiduddin[1,2], Juhee Pae[2], Ruth Lehmann[11]Whitehead Institute, [2]NYU Grossman School of Medicine

Primordial germ cells (PGCs) are the first cells to form in the early *Drosophila* embryo. This cellularization event involves two cytokinesis events. The first is the anaphase furrow, which is spindle-dependent, which is required to cleave one pole bud into two. The second is the bud furrow, which is spindle-independent, and which is required to form the PGC and separate it from the rest of the syncytial embryo. This process requires a ubiquitin ligase adaptor Germ-cell-less (GCL), which has been shown to degrade Torso, a receptor tyrosine kinase. When GCL is not maternally deposited in the embryo, pole buds are still able to divide at the anaphase furrow, but fail to constrict at the bud furrow, and so PGC formation fails to occur. Without suppression by GCL, Torso activates Ras signaling. Later in embryogenesis, Ras is known to initiate the Raf/MEK/MAPK signaling cascade, leading to the transcriptional upregulation of somatic terminal genes. However, at the time of PGC formation, the embryo is still transcriptionally silent, and relies entirely on the maternal contribution of RNAs and proteins. Therefore, Ras acts in a non-canonical transcription-independent way to antagonize the formation of PGCs. Our data suggests that when GCL is not present, Ras inappropriately activates somatic cues that affect membrane dynamics and the distribution of cytokinetic ring components to prevent PGC formation.

126 **Evolutionarily Conserved Regulators of Muscle Type-Specific Mitochondrial Network Organization** Prasanna Katti[1], Brian Glancy[21]National Institutes of Health/ NHLBI, [2]National Heart, Lung, and Blood Institute

Muscle mitochondria form highly organized muscle type-specific networks that facilitate energy distribution and intracellular signaling. Mitochondrial network organization is altered during stress, aging, and other pathophysiological states. However, pathways regulating muscle type-specific mitochondrial size, content, and network organization across different muscle types remain unclear. We demonstrated that Drosophila muscles exhibit distinct mitochondrial networks, similar to mouse cardiac and oxidative muscles, and display varying sarcoplasmic reticulum content depending on muscle type, as seen in vertebrate muscles. Knockdown of regulators of mitochondrial dynamics, *Marf* (mammalian orthologs: Mfn1/2), *Drp1 (Dnm1/2)*, and *Fis1*, and knockdown of mitochondrial transport factor, Miro, affected mitochondrial size, but not mitochondrial network organization. Further, the knockdown of muscle fiber-

type specification factor, *salm (Sall1-4)*, in adult Drosophila indirect flight muscles altered muscle contractile type and mitochondrial network organization. Proteomic analysis of Drosophila jump, leg, and indirect flight muscles and muscles misexpressing *salm* identified transcription factors *H15* and cut as potential regulators of mitochondrial organization. RNAi knockdown studies revealed that *H15 (Tbx15,20)* and *cut (Cux1-2)* regulate mitochondrial content and organization independent of muscle-contractile type in jump and leg muscles. Further, cut overexpression suppressed salm expression in flight muscles and resulted in tubular muscle type indicating that cut regulates fiber type specification in fibrillar flight muscles upstream of salm. These results establish that muscle contractile type, mitochondrial network configuration, mitochondrial content, and individual mitochondrial size can each be modulated independently of the other parameters. We show that cut and H15, acting upstream and downstream of salm, respectively, regulate mitochondrial network configuration independent of muscle contractile type.

127 ***SPTSSA* associated hereditary spastic paraplegia: modeling the disease and screening for drugs in flies** Xueyang Pan[1,2], Siddharth Srivastava[3], Hagar Mor Shaked[4], Kenneth Gable[5], Sita D Gupta[5], Niranjanakumari Somashekarappa[5], Gongshe Han[5], Payam Mohassel[6], Marc Gotkine[4], Elizabeth Doney[7], Paula Goldenberg[8], Queenie K.-G Tan[9], Yi Gong[10,11], Benjamin Kleinstiver[11,12,13], Brian Wishart[8], Heidi Cope[9], Claudia Brito Pires[10,11], Hannah Stutzman[11,12], Rebecca C Spillmann[9], Undiagnosed Disease Network[14], Reza Seyedsadjadi[10], Orly Elpeleg[4], Chia-Hsueh Lee[15], Simon Edvardson[16], Florian Eichler[10,11], Teresa M Dunn[5], Hugo J Bellen[1,21]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, [3]Department of Neurology, Boston Children's Hospital, Harvard Medical School, [4]Department of Genetics, Hadassah Medical Center and Faculty of Medicine, Hebrew University of Jerusalem, [5]Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, [6]Department of Neurology, Johns Hopkins University, [7]Massachusetts Eye and Ear, [8]Department of Pediatrics, Massachusetts General Hospital, [9]Department of Pediatrics, Duke University School of Medicine, [10]Department of Neurology, Massachusetts General Hospital, [11]Center for Genomic Medicine, Massachusetts General Hospital, [12]Department of Pathology, Massachusetts General Hospital, [13]Department of Pathology, Harvard Medical School, [14]Undiagnosed Disease Network, [15]Department of Structural Biology, St. Jude Children's Research Hospital, [16]Pediatric Neurology Unit, Hadassah University Hospital, Mount Scopus

Human *SPTSSA* encodes an activating subunit of serine palmitoyltransferase (SPT), an enzyme complex of three subunits that catalyzes the rate-limiting step of sphingolipid (SL) *de novo* synthesis. SLs are a diverse family of lipids with critical structural and signaling functions in the nervous system. The synthesis of SLs is tightly regulated by ORMDL proteins that bind to SPT and mediate a feedback inhibition of SPT enzymatic activity when ceramide levels become elevated. To date, no Mendelian disorder has been associated with *SPTSSA*. We identified three unrelated individuals with variants in *SPTSSA* who display a severe and complex form of hereditary spastic paraplegia (HSP) with manifestations including early-onset progressive motor impairment and spasticity, variable sensorineural hearing loss and language/cognitive dysfunction. The cryo-EM structure of the human SPT/ORMDL3 complex reveals that the patient *SPTSSA* variants affect residues that reside in close association with ORMDL3, indicating their importance in the interaction between SPT and ORMDL3. Using biochemical and cell-based assays, we showed that patient variants impaired the negative regulation of ORMDL and led to excessive SL synthesis. Increased SPT activity is evident based on the elevated SLs in serum and fibroblasts from patients. We modeled the disease using fruit fly by overexpressing the three human SPT subunits as a fusion protein and showed that SPT overexpression led to excessive SL synthesis and caused severe motor defects and shortened lifespan, supporting the causal relationship between elevated SPT activity and some of the patient phenotypes. Moreover, while co-expression of human ORMDL3 fully reversed the elevated SL levels and rescued the phenotypes in flies expressing the wildtype SPT complex, it failed to fully rescue flies expressing the mutant SPT containing *SPTSSA* p.Thr51Ile variant, confirming that the variant abrogates ORMDL regulation of SPT *in vivo*. Treatment with myriocin, a SPT inhibitor, extended the lifespan of the SPT overexpression flies, suggesting that SPT inhibitors may be considered as reagents for the treatment of the disease. A screen for novel SPT inhibitors is in progress. In summary, we identified *SPTSSA* as a novel gene associated with HSP, modeled the disease using fruit flies, and discovered a potential treatment strategy for the disease.

128 **Suppression of phototactic tendencies in flies as a learning assay for models of cognitive decline** Giovanna Marie Crystal Novi[1], S. Tariq-Ahmad[21]Colby College, [2]Biology, Colby College

Drosophila are a well-known organism widely used to model various categories of diseases, especially because of the possibility of genetic manipulation and their inexpensive maintenance. By being short-lived, *Drosophila* allows for fast data collection, which would take years in other models. The advantages of using this model are even more profound in the context of neurodegenerative diseases and dementia. Nevertheless, *Drosophila* models of dementia can face the

criticism of being unable to effectively measure complex behavior or cognitive decline.

Although there are other models using associative learning as a proxy for cognitive decline, they present some drawbacks. Most models use shock as an aversive unconditioned stimulus, which requires a more complex setup. We adapted an associative learning assay to be practical and cost-effective, with an easier set up. We used quinine as an aversive stimulus to suppress *Drosophila*'s phototactic tendencies. We placed 10 to 15 flies in two connected vials: one of the vials was open to light, while the other was kept dark. We placed quinine in the light vial and performed 16 training trials with the flies. After training, flies trained with quinine showed reduced phototactic tendencies. Flies trained with water did not show reduced phototaxis. Signs of cognitive decline would be shown if flies fail to associate the aversive stimulus with light, maintaining the phototactic tendencies.

The majority of *Drosophila* models of Alzheimer's disease is genetic. As a primary model for cognitive decline, we decided to validate a chemically induced model of Alzheimer's disease. Aluminum is commonly used as a chemical to induce Alzheimer's disease in vertebrates, and we wanted to see if this model would be effective in inducing Alzheimer's in *Drosophila*. We administered aluminum to flies, and found reduced suppression of phototactic tendencies, which suggests failure in associative learning. These results demonstrate that our adapted associative learning assay works as a proxy for cognitive decline in *Drosophila*.

Being able to assess learning in fruit flies will allow for the measurement of complex behaviors and cognitive decline, making this organism an even more powerful model for the multiple causes of dementia.

129 **The Y225A substitution induces long-range conformational changes in human PrP that are protective in *Drosophila*** Pedro Fernandez-Funez[1], Ryan Myers[1], Aliciarose John[2], Alessandro Cembran[31]Biomedical Sciences, University of Minnesota Medical School, [2]University of Minnesota Duluth, [3]Chemistry and Biochemistry, University of Minnesota Duluth

The prion protein (PrP) is an enigmatic protein responsible for devastating neurological disorders called prion diseases. PrP is a glycoprotein with a small globular domain that can misfold and form toxic aggregates. An unresolved problem in PrP biology is identifying the mechanisms governing PrP conformational dynamics, misfolding, and toxicity. To answer that questions, we investigate the mechanisms linking PrP sequence and structure to disease susceptibility in animals. In previous work, we learned that flies expressing PrP from animals resistant to prion diseases (horse, dog, rabbit) show no toxicity. In contrast, flies expressing mouse, hamster, or human PrP show progressive toxicity, with human PrP being the most toxic. We leveraged this knowledge to identify the residues governing human PrP dynamics and toxicity. Our unique approach consists of examining the impact of PrP substitutions using molecular dynamics (MD) simulations along with toxicity and aggregation studies in flies. We focused our attention on a PrP subdomain consisting of the distal helix 3 and the b2-a2 loop, the C-terminal 3D domain (CT3DD), known to play a key role in PrP misfolding. Here, we describe the impact of replacing Y225 in human PrP with A225 from rabbit PrP (Y225A). MD simulations show that in PrP-WT the b2-a2 loop populates 6 different conformations separated by low energy barriers, 5 of which containing b-turns. In contrast, in Y225A the b2-a2 loop populates mostly a 3_{10}-turn with lower fluctuations and hydrophobic exposure, mainly the result of stacking Y169 and F175. Next, we created transgenic flies and found that expression of PrP-Y225A rescues the glassy eye induced by PrP-WT. Detailed studies in brain neurons show that PrP-WT induces abnormal differentiation and progressive degeneration of the axonal projections of the mushroom bodies along with intracellular PrP accumulation and expansion of the cell body clusters. Expression of PrP-Y225A shows better preservation of axonal projections and now expansion of the cell body clusters. Lastly, PrP-Y225A shows lower accumulation of insoluble PrP in insolubility assays. Overall, Y225A promotes the population of a structured loop that increases the stability of the globular domain and lowers toxicity in vivo. The innovative combination of *in silico* and *in vivo* approaches identified novel molecular mechanisms regulating PrP dynamics and toxicity along with the key role of other residues in the loop (165-175).

130 **A novel model for the *in vivo* screening of small ingestible compounds with anti-amyloid-β properties** Rosalind Heron[1], Robert Williams[2], Will Wood[11]University of Edinburgh, [2]University of Bath

Flavonoids, which have numerous health benefits, are of growing interest to neuroscientists thanks to their potential to lower the risk of developing Alzheimer's disease (AD) and dementia. In animal models, some flavonoids are able to reduce the levels of toxic amyloid associated with AD and, although ongoing clinical trials remain in the early stages, they show similar promise. To help understand the ability of flavonoids to clear toxic amyloid-*beta* and to aid in the development of suitable interventions for AD, we utilise a genetically versatile *Drosophila melanogaster* model of AD that replicates the overproduction and secretion of toxic amyloid-*beta* (hAβ42) from neurons. This AD model presents

distinctive behavioural phenotypes such as locomotor defects and early mortality. Immunofluorescent staining shows that these phenotypes are associated with characteristic hAβ42 accumulation in subsets of neurons. By feeding different flavonoids to these animals in a blinded and statistically powerful study, we have undertaken structure-activity relationships and observed varying abilities of flavonoids to reduce the levels of hAβ42 around the neurons and prevent locomotor defects. All animals were age-matched and sex differences were assessed in this study. Notably, when neuronal clearance of hAβ42 was partial, the locomotor rescue was correspondingly weaker. In conclusion, we have developed a genetically tractable and versatile *in vivo* model of AD that allows us to quickly explore the mechanisms through which ingested flavonoids can exert anti-amyloid activity in AD and support further development of flavonoid-based therapeutics.

131 **Septins regulate heart contractility through changes in activity of cardiomyocyte store-operated Ca^{2+} entry.** Ben A Tripoli[1], Courtney E Petersen[2], Jeremy Smyth[11]APG, Uniformed Services University of the Health Sciences, [2]National Institutes of Health

Proper calcium handling in cardiomyocytes is paramount to maintaining cardiomyocyte contractility and heart function. Recent data from our lab and others in the field have established that store operated calcium entry (SOCE) is an essential component of cardiomyocyte calcium regulation. SOCE refers to calcium influx that is activated by depletion of endo/sarcoplasmic reticulum (E/SR) calcium stores. The pathway is mediated by STIM proteins, which act as calcium sensors in the E/SR, and ORAI calcium influx channels in the plasma membrane. Both upregulation and suppression of SOCE in cardiomyocytes result in cardiomyopathy, demonstrating that proper regulation of cardiomyocyte SOCE is essential. However, the mechanisms responsible for SOCE regulation in cardiomyocytes are not well understood. To this end, septin GTPases have emerged as regulators of SOCE in non-cardiac tissues, with septin 1, 2, or 4 suppression resulting in SOCE suppression and septin 7 suppression resulting in SOCE upregulation. Importantly, the role of septins in cardiomyocytes is nearly completely unknown. Through intravital imaging analysis of *Drosophila* heart contractility, we now show that cardiomyocyte specific RNAi-based depletion of septins 1, 2, or 4 results in dilated cardiomyopathy nearly identical to that caused by SOCE suppression. Co-expression of septin 2 RNAi with a constitutively active Orai channel reverses the septin 2 phenotype, strongly suggesting that the septin 2 phenotype is due to SOCE suppression. In addition, overexpression of SERCA, which pumps Ca^{2+} into SR stores, also reversed the septin 2 phenotype, further implicating a mechanism whereby septin depletion reduces SR Ca^{2+} store refilling due to suppressed SOCE. Interestingly, septin 7 suppression resulted in hypertrophic cardiomyopathy similar to that caused by SOCE upregulation, and micro-computerized tomography (microCT) analysis of heart size further confirmed the hypertrophic phenotype of septin 7 depleted hearts. This septin 7 phenotype was reversed by Orai depletion, again supporting a role for SOCE dysregulation in the septin 7 depletion-mediated cardiac phenotype. These results demonstrate for the first time an essential role for septins in cardiomyocytes, and our ongoing studies are investigating the mechanisms by which septins regulate SOCE in these highly specialized cells.

132 **Neuronal and molecular mechanisms of Nf1-dependent metabolic regulation** Valentina Botero[1,2], Eliza Grenci[1], Tamara Boto[3], Scarlet Park[1,4], James Walker[5,6], William Ja[4], Seth Tomchik[21]Scripps Research Institute, [2]University of Iowa, [3]Trinity College Dublin, [4]UF Scripps Biomedical Research, [5]Massachusetts General Hospital, [6]Harvard Medical School

Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutations in the *NF1* gene. *NF1* encodes neurofibromin (Nf1), a large protein that functions as a negative regulator of Ras signaling and mediates pleiotropic organismal and cellular functions. Patients with NF1 are predisposed to a range of complications, including benign tumor formations in the nervous system, increased rate of cognitive and developmental disorders, altered cellular function, short stature, and shorter lifespan. Emerging data suggest that Nf1 regulates metabolism: NF1 patients show a reduced body mass index, alterations in metabolites, lower incidences of diabetes, and increased resting energy expenditure. These changes in metabolism may contribute to complications and symptoms associated with NF1. The mechanisms by which Nf1 affects metabolism and energy expenditure are not well understood. Using the *Drosophila melanogaster NF1* ortholog, we show that Nf1 regulates metabolic homeostasis via neuronal mechanisms. *Drosophila* Nf1 is ~60% identical to the human protein and similarly mediates Ras signaling. Our data show that the loss of Nf1 increases metabolic rate via a Ras-GAP-related domain, increases metabolic rate, feeding rate, starvation susceptibility, and alters lipid stores and turnover kinetics. These metabolic alterations map to a restricted subset of neurons in the ventral nervous system and are independent of activity. Activation of this restricted neuronal circuit mimics the loss of Nf1 function by increasing metabolic rate when stimulated. Data suggest that Nf1 regulates metabolic rate via key signaling pathways in neurons, which act on specific peripheral tissues to regulate metabolism. These data indicate that Nf1 may regulate changes in

neuronal metabolic control, suggesting that cellular and systemic metabolic alterations may be a pathophysiological mechanism of NF1, and provide a platform for investigating the cellular role of Nf1 in metabolic homeostasis.

133 **Moira, a component of Drosophila SWI/SNF complex, compacts circadian chromatin to enable ~24-hour circadian rhythms** Swathi Yadlapalli[1], Qianqian Chen[2], Ye Yuan[21]Cell and Developmental Biology, University of Michigan, [2]University of Michigan

Circadian clocks in all eukaryotes are based on negative transcriptional translational feedback loops. Using CRISPR-Cas9 generated knock-in flies and high-resolution live imaging, our lab has recently shown that *Drosophila* clock proteins, PERIOD and CLOCK, are organized into a few (~10) dynamic, non-membrane-bound nuclear condensates positioned close to the inner nuclear envelope in clock neurons during the circadian repression phase (Xiao et al, PNAS 2021). More recently, our studies revealed that clock protein and chromatin complexes are assembled within these nuclear condensates to enable circadian gene repression. What is the mechanism through which clock protein condensates enable circadian gene repression? To address this question, it is critical to identify the proteins that colocalize to the clock protein condensates. Towards this goal, we generated endogenously tagged PERIOD-miniTurboID flies and performed 10-plex tandem mass tag (TMT10) -based quantitative proteomic experiment. Our experiments revealed that Moira, a component of *Drosophila* SWI/SNF chromatin remodeling complex and a homolog of human BAF155, and other SWI/SNF components are key interaction partners of PERIOD during the circadian repression phase. By fluorescent tagging of endogenous Moira protein, strikingly, we find that Moira is organized into nuclear condensates at the nuclear periphery throughout the circadian cycle, and these condensates colocalize with PERIOD condensates specifically during the repression phase in clock neurons. Using ATAC-seq and RNA-seq on FAC-sorted clock neurons we found that Moira is required for chromatin compaction of clock-regulated genes during the repression phase, and knockdown of Moira results in complete de-repression of clock-regulated genes leading to 100% behavioral arrhythmicity. Our results demonstrate that Moira-mediated chromatin compaction constitutes a critical first step in the repression of clock regulated genes and for generating ~24-h circadian rhythms.

134 **Maintenance of Terminal Differentiation by Retinoblastoma and Hippo Tumor Suppressors** Alexandra Rader, Battuya Bayarmagnai, Maxim FrolovUniversity of Illinois at Chicago

Both the Retinoblastoma (pRB) and Hippo tumor suppressor pathways are necessary for the appropriate specification of countless cell types and are frequently dysregulated in cancer. Our lab has previously demonstrated that during Drosophila eye development *Rbf* and *warts* double mutant photoreceptor neurons properly differentiate but fail to maintain neuronal specification. Here, we propose a mechanism by which the pRB and Hippo pathways converge to protect photoreceptor neurons from dedifferentiation. We performed single-cell RNA-sequencing on wildtype, *Rbf* single, *wts* single and *Rbf wts* double mutant eyes to identify dedifferentiated photoreceptor neurons and understand the transcriptional changes accompanying loss of neuronal identity. These experiments revealed that dedifferentiated neurons aberrantly express *homothorax* (*Hth*), a Hox transcription factor and suppressor of differentiation whose expression is normally restricted to progenitor cells.We then performed genetic experiments in eye tissues expressing constitutively active *yorkie*, a downstream effector of Hippo signaling, and showed that ectopic expression of *Hth* is both necessary and sufficient for photoreceptor dedifferentiation. Interestingly, we found that a known Rbf binding protein, the GAGA transcription factor (GAF), is required to prevent Yki dependent activation of Hth and photoreceptor dedifferentiation. We further show that Yki and Rbf physically interact and can regulate the *Hth* reporter in tissue culture cells. We suggest that Rbf, presumably through interaction with GAF, prevents Yki from inappropriate activation of Hth to keep photoreceptors in the state of terminal differentiation.

135 **Coordinating stereotyped and stochastic patterns in the *Drosophila* eye** Alison Ordway, Caitlin Anderson, Lukas Voortman, Elizabeth Urban, Robert JohnstonBiology, 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. Underlying 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.

Cubitus Interruptus (Ci), an effector of Hh signaling, binds to an eye specific enhancer in *ss*. This site overlaps with a binding site for Klumpfuss (Klu), a repressor of *ss*, suggesting competitive binding and regulation. 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.

136 **Blimp-1 and Hr3/RORβ specify the blue-sensitive photoreceptor subtype in *Drosophila* by repressing the Hippo pathway** Mhamed Bashir[1], Joseph Bunker[1], Sydney Bailey[1,1], Alexis Perry[1], Simon Sprecher[2], Gerald B. Call[3], Jens Rister[11]University of Massachusetts Boston, [2]University of Fribourg, [3]Midwestern University

During terminal differentiation of the mammalian retina, transcription factors control binary cell fate decisions that generate functionally distinct subtypes of photoreceptor neurons. For instance, Otx2 and RORβ activate expression of the transcriptional repressor Blimp-1/PRDM1 that represses bipolar interneuron fate and promotes rod photoreceptor fate in the mammalian retina. Moreover, Otx2 and Crx promote expression of the nuclear receptor Nrl that promotes rod photoreceptor fate and represses cone photoreceptor fate. Mutations in these four transcription factors cause severe eye diseases such as retinitis pigmentosa. Here, we show that a post-mitotic binary fate decision in Drosophila color photoreceptor subtype specification requires ecdysone signaling and involves orthologs of these transcription factors: Drosophila Blimp-1/PRDM1 and Hr3/RORβ promote blue-sensitive photoreceptor fate and repress green-sensitive photoreceptor fate through the transcriptional repression of warts/LATS, the nexus of the phylogenetically conserved Hippo tumor suppressor pathway. Moreover, we identify a novel genetic interaction between Blimp-1 and warts, whereby Blimp-1 represses a warts intronic enhancer in blue-sensitive photoreceptors, giving rise to green-sensitive photoreceptor specific expression of warts. Together, these results reveal conserved transcriptional regulators that play key roles in terminal cell fate decisions in both the Drosophila and mammalian retina, and mechanistic insights further deepen our understanding of how Hippo pathway signaling is repurposed to control this cell fate decision in Drosophila.

137 **Uncovering the role of protein quality control systems in shaping tissue-specific proteomes** Kai Zhang[1], Rajan Burt[2], Ying Zhang[1], Yan Hao[1], Paulo Leal[1], Kausik Si[1,31]Stowers Institute for Medical Research, [2]University of Kansas Medical Center, [3]Department of Molecular and Integrative Physiology, University of Kansas Medical Center

Tissue-specific proteome is determined by differential protein synthesis and breakdown. Compared to selective gene expression, however, it is largely unknown what is the role of protein degradation in forming the cell-type-specific proteome. Recently, we have observed that there are tissue-specific biases in utilization of various protein quality control (PQC) systems, including ubiquitin-proteasome system, autophagy, and mitochondrial degradation pathway. To investigate if PQC systems degrade tissue-specific substrates, we take ubiquitination as a maker of protein degradation and applied mass spectrometry approaches to identify ubiquitinome changes upon perturbation of various PQC systems in different fly tissues. We find that each tissue has common as well as unique ubiquitinome and perturbation of a specific protein quality control system lead to tissue-specific changes in protein ubiquitination. These datasets positioned us to examine whether PQC systems sculpt tissue-specific proteome in an mRNA or protein expression-independent manner. We are comparing the ubiquitinome dataset with transcriptome and TMT-quantified proteome for each tissue to identify proteins that are regulated in a tissue-specific manner by PQC systems. In addition to providing a new function of PQC systems in tissue-identity, this analysis may help us to understand why mutations on PQC systems can lead to tissue-specific defects.

138 **Pervasive SUMOylation of piRNA pathway factors revealed by diGly proteomics** Maria Ninova[1], Hannah Holmes[2], Katalin Fejes Toth[3], Alexei Aravin[31]Biochemistry, UC Riverside, [2]UC Riverside, [3]California Institute of Technology

Piwi proteins and Piwi-interacting RNAs (piRNAs) play a central role in the silencing of transposable elements (TEs) in animal germlines and are essential for fertility. In *Drosophila*, the germline piRNA pathway involves intimately linked processes of piRNA biogenesis, and TE co-transcriptional and post-transcriptional silencing: In a specialized perinuclear structure called nuage, piRNA-associated Piwi proteins Aub and Ago3 engage in a 'ping-pong' mechanism that cleaves TE RNAs and fuels further piRNA production; a portion of TE-antisense piRNAs get loaded into a third protein, Piwi, which then enters to the nucleus to find TE nascent RNAs and enforce repressive chromatin formation at target loci. Genetic screens identified a myriad of piRNA pathway players, including SUMO – a small protein that gets conjugated to lysine side chains of target proteins and serves as a post-translational modification with various regulatory outcomes. Recent work identified several SUMO-dependent processes within the nuclear piRNA pathway related to heterochromatin formation at TE targets. However, despite this progress, our understanding of the piRNA pathway molecular mechanisms

and the role of SUMOylation in this context is still far from complete.

Protein modification by SUMO has remained understudied due to its typically transient nature and incompatibility with standard proteomics approaches. To address this challenge, we developed a transgenic system and adapted a diGly proteomics-based method which for the first time enabled unbiased detection of SUMOylation sites with aminoacid-level resolution in the Drosophila ovary (**Ninova et al. 2022 *biorXiv***), leading to the identification of over ~1500 SUMO sites in over 800 proteins. Strikingly, the topmost overrepresented functional categories within the 'SUMOylome' include factors involved in TE silencing. In addition to general chromatin proteins, we discovered SUMO sites at proteins specific to the piRNA pathway including the core component Piwi, and multiple additional nuclear and unexpectedly, nuage-associated factors. We further found that SUMO depletion disrupts the integrity of the nuage, and that SUMOylation of two nuage-localizing proteins, Maelstrom and Spindle-E, is Piwi-dependent. Collectively, these findings suggest a broad and multifaceted role of protein SUMOylation in the piRNA pathway that extends beyond currently known functions and open important new frontiers for future research.

139 **Actomyosin contraction in follicular epithelium is the major mechanical force for follicle rupture during *Drosophila* ovulation** Stella Cho[1], Wei Li[2], Risa Kiernan[2], Jianjun Sun[2,31]Physiology and Neurobiology, University of Connecticut, [2]University of Connecticut, [3]Institute for Systems Genomics

Ovulation is critical for sexual reproduction and consists of the process of liberating fertilizable oocytes from their somatic follicle capsules, also named follicle rupture. The mechanical force for this liberation or follicle rupture is largely unknown in many species. Our previous work demonstrated that *Drosophila* ovulation, as in mammals, requires the proteolytic degradation of the posterior follicle wall and the follicle rupture to release the mature oocyte from a layer of somatic follicle cells. Here, we explored the mechanical force for this rupture using our novel *ex vivo* follicle rupture assay. Pharmacological analysis suggests that the nature of this force is not osmotic swelling of the oocyte during follicle rupture or tubulin-mediated mechanical force, but actin-mediated mechanical force. Mature follicles treated with cytochalasin D or latrunculin A, two chemicals blocking F-actin polymerization, did not respond to octopamine (OA)-induced follicle rupture. Strikingly, OA stimulation led to drastic enrichment of F-actin and non-muscle myosin II (NMM II) in the cortex of the mature follicle cells, consistent with our hypothesis that actomyosin contraction from the follicle cells is the major mechanical force for follicle rupture. In addition, we demonstrated that OA-induced F-actin and NMM II cortex enrichment relies on the octopamine receptor in mushroom body (Oamb) in follicle cells. We also showed that OA induces Rho1 GTPase (Rho1) activation in the follicle cell cortex. Lastly, blocking Rho kinase (Rok) or Rho1, two important upstream regulators for actomyosin contraction, inhibited OA-induced follicle rupture *ex vivo* and ovulation *in vivo*. All these results led us to conclude that OA/Oamb induces actomyosin cortex enrichment and contractility, which generates the mechanical force for follicle rupture during *Drosophila* ovulation. Due to the conserved nature of actomyosin contraction, this work could shed light on the mechanical force for follicle rupture in other species including humans.

140 **Localization of mRNA encoding the actin-binding protein Sry-α promotes nuclear repositioning** Tejas Mahadevan[1], Lauren Figard[2], Anna Sokac[11]Cell and Developmental Biology, University of Illinois Urbana Champaign, [2]verna and mars mclean department of biochemistry and molecular biology, Baylor College of Medicine

Nuclear repositioning occurs in many cell types and contexts, including skeletal muscle cells (Roman and Gomes, 2018) and motile fibroblasts (Gundersen and Worman, 2013). However, we have limited understanding of the players involved in nuclear repositioning; and our understanding is even less for nuclei that are repositioned in cells during morphogenesis. The *Drosophila* embryo develops as a syncytium, undergoing 13 successive nuclear divisions without intervening cytokinesis (Sokac et al., 2022). After the 13th nuclear division, the embryo initiates its first morphogenetic event, called cellularization. During cellularization, approximately 6000 nuclei are packaged into individual cells that make an epithelial sheet. We found that these nuclei move away from the plasma membrane to a more basal position during late cellularization. We also found that knocking out the actin filament (F-actin) binding protein Serendipity-α (Sry-α) leads to improper nuclear positioning during late cellularization. Specifically, the nuclei fail to reposition and are located too close to the plasma membrane in *sry-α* mutants. Through live imaging, the loss of Sry-α appears to compromise a force-balance required for normal nuclear positioning. On further inspection, we saw that the mRNA encoding *sry-α*, and the protein itself, are localized during late cellularization in a cloud around the centrosomes, which are tethered to the nuclei. Using point mutations of *sry-α* that encode wild-type protein but whose mRNA fails to localize to the centrosomal clouds we have determined that *sry-α* mRNA apical localization is required for nuclear repositioning during late cellularization. We suggest that proper nuclear repositioning during late cellularization, facilitated by apical *sry-α* mRNA and/or protein, is necessary to shield nuclei from squeezing due to apical forces during gastrulation which

immediately follows cellularization. We believe that *sry-α* achieves this by coordinating interactions between actin and microtubules to reposition the nuclei during late cellularization.

141 **Determining the roles of Prostaglandins and Fascin in regulating nuclear architecture during collective cell migration** Tina L Tootle[1], Ashley Goll[21]University of Iowa, [2]Anatomy and Cell Biology, University of Iowa

Cell migration is required for development, wound healing, and cancer. During migration, the cytoskeleton rearranges to polarize the cell, and for 3D migration, the nucleus must deform. The nucleus is the largest organelle within the cell and limits the ability of cells to migrate through restrictive areas. The nucleus receives information about the external environment through the Linker of the Nucleoskeleton and Cytoskeleton (LINC) Complex which drives alterations in nucleoskeletal composition to regulate nuclear stiffness. If there is more Lamin A and Emerin the nucleus is stiffer, while an increase in Lamin B results in a softer nucleus. While LINC Complex-mediated nucleoskeletal changes occur during 3D single cell migration, it is not known whether this is required during 3D collective cell migration. To address this unknown, we use the *in vivo*, 3D collective cell migration of the Drosophila border cells as a model. We find that Lamin B is enriched in both the border and polar cells, whereas Lamin A is restricted to the polar cells throughout migration. Emerin is dynamic during migration; it is prominent in both the border cells and polar cells at the beginning of migration but becomes restricted to the polar cells at mid-migration. Having found the nucleoskeleton is dynamic during border cell migration, we next asked if this is regulated by prostaglandin (PG)-dependent regulation of Fascin. PGs are short range lipid molecules that are required for on-time border cell migration. One downstream target of PGs is Fascin. Fascin is not only an actin bundling protein but also acts as an adaptor between the LINC Complex and the actin cytoskeleton to facilitate the transmission of mechanical signaling to the nucleus during single cell migration. Thus, we asked whether PGs and Fascin regulate nucleoskeletal composition during border cell migration. We find that loss of PGs or Fascin increases Lamin A and Emerin prevalence at the nuclear membrane in the border cells throughout migration, suggesting increased nuclear stiffness. As border cell migration is delayed when PGs or Fascin are lost, these findings suggest nuclear stiffness is a limiting factor in border cell migration. Together, these results suggest that PGs and Fascin are required for the transmission of mechanical signaling from the external environment, through the cytoskeleton, to the nucleus necessary for invasive, collective cell migration.

142 **Recruitment of transcription machinery to HLBs during Drosophila embryogenesis occurs independently of histone gene transcription** James P Kemp Jr[1], Mia Hoover[2], Chun-Yi Cho[3], Patrick H O'Farrell[3], William F. F Marzluff[4], Robert J Duronio[51]Integrative Program for Biological and Genome Sciences, University of North Carolina Chapel Hill, [2]Department of Biology, University of North Carolina Chapel Hill, [3]Department of Biochemistry and Biophysics, University of California San Francisco, [4]Integrative Program for Biological and Genome Sciences, Department of Biology, Department of Biochemistry and Biophysics, University of North Carolina Chapel Hill, [5]Integrative Program for Biological and Genome Sciences, Department of Biology, Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, Department of Genetics, University of North Carolina Chapel Hill

Nuclear bodies are membrane-less biomolecular condensates associated with production and processing of RNAs within the nucleus. Understanding how the recruitment of factors controls transcription within nuclear bodies is of great interest, but the mechanisms involved remain to be elucidated. We study these questions using Drosophila Histone Locus Bodies (HLBs). HLBs are phase separated, micron sized nuclear bodies that form at replication dependent (RD) histone genes and contain all factors necessary for transcription and 3' end processing of RD histone mRNA. Using a combination of live imaging and fixed tissue immunofluorescence of endogenously tagged proteins in early embryos, we examined the dynamics of RNA Pol II recruitment to the HLB and its correlation with transcription of RD histone genes. In the syncytial embryo, the HLB forms coincidently with the appearance of nascent RD histone transcripts in cycle 11. By functionally inactivating maternally deposited nuclear proteins using "Jabba Trap", we found that RNA Pol II and the scaffolding protein MXC were both required for full HLB formation. Surprisingly, throughout embryogenesis RNA Pol II is always present in the HLB irrespective of cell cycle phase, including in G1 arrested cells not transcribing RD histone genes. This finding indicates that RNA Pol II recruitment to the HLB can occur independently of RD histone gene transcription. In addition, using RNA FISH to detect nascent RD histone transcripts within the HLB, we found that after initiation in early interphase RD histone genes remain transcriptionally active during S and G2 phases of the first 16 cycles of embryogenesis. We also examined the behavior of the elongation factor SPT6 during RD histone gene transcription. Like RNA Pol II, SPT6 is present in the HLB coincidently with RD histone gene transcription throughout interphase of syncytial blastoderm cycles 11-13. However, in contrast to RNA Pol II and nascent RD histone transcripts, SPT6 is only transiently present in the HLB during the first few minutes of interphase 14 as the embryo cellularizes. This result suggests that SPT6 is not required for ongoing transcription elongation after initiation following mitosis 13. Finally,

we are determining how pioneering factors like CLAMP facilitate initial formation of the HLB and transcription of the RD histone genes. Our findings point to a separation of function between recruitment and activity of transcriptional machinery to nuclear bodies.

143 **Dysregulation of the ER blocks recruitment of centrosome associated proteins, resulting in mitotic failure**
Katherine R Rollins, J Todd BlankenshipBiological Sciences, University of Denver

The Endoplasmic Reticulum makes contacts with organelles throughout the cell and has recently been shown to participate in processes beyond its canonical roles of calcium storage and protein synthesis. The ER also undergoes a remarkable transition in morphology during cell division, with mitotic divisions requiring a massive remodeling of various membranous organelles such as the nuclear envelope and the associated ER. However, whether (and how) changes in ER behaviors modulate mitotic events is less clear. The rapid cleavage divisions in the early *Drosophila* embryo are a potent system to understand the dynamic changes that underlie successful mitotic events, as well as uncover the distinct challenges that occur during the rapid cleavage cycles that exist in many animal embryos. Here, we examine the behaviors and contributions of the ER to mitotic function during the cleavage divisions. As the mitotic spindle begins to form, the ER retracts and clusters around the spindle poles as well as forming a coated structure around the spindle. In a screen for Rab family GTPases that display dynamic function at these stages, we identified Rab1. Disrupting Rab1 function in the early embryo led to an enhanced buildup of ER at the spindle poles and, interestingly, produced deep defects in the division cycles. Mitotic spindle assembly was disrupted and an intriguing “mini-spindle” phenotype emerged. We next explored two models for how the ER might drive these defects— either through a physical constraint model or a decreased recruitment of nucleators model. Our results further distinguish between these two models and suggests that the overaccumulation of ER can suppress the recruitment of key centrosome proteins to the spindle poles leading to reduced γ-tubulin function. Finally, we show that the division failures and ER spindle-pole accumulation in *Rab1* knockdown embryos can be rescued with a Dynein inhibitor, demonstrating that Dynein motor activity is essential for the spindle recruitment of the ER during mitosis. These results suggest that ER levels must be carefully tuned during mitotic processes to ensure proper assembly of the division machinery.

144 **Regulation of Hippo signaling by the “tug of war” between apical polarity and actomyosin dynamics**
Sherzod A Tokamov[1], Nicki Nouri[2], Richard Fehon[21]Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, [2]Molecular Genetics and Cell Biology, University of Chicago

How organs achieve their optimal size is a fundamental question in developmental biology. Organs are lined with polarized epithelia, and epithelial tissue growth is a key determinant of organ size. First discovered in *Drosophila*, the Hippo pathway is now recognized as a major signaling pathway regulating tissue growth. At the core of the Hippo pathway, a kinase cascade inhibits a transcriptional co-factor, an oncoprotein called Yorkie. Upstream of this kinase cascade, Kibra functions as a multivalent scaffold protein that localizes at the junctional and apicomedial cortex of epithelial cells and promotes pathway activation by assembling pathway components. Previous work in our lab showed that localization at the apicomedial cortex potentiates Kibra-mediated signaling. However, how Kibra is organized and regulated at the cell cortex remains unknown. Here we demonstrate that Kibra localization and function are controlled by competition between the apical polarity network and actomyosin dynamics. We find that while apicomedial actomyosin flows promote Kibra accumulation at the medial cortex, the apical polarity network tethers Kibra at the junctional cortex. Our results suggest a model whereby a ‘tug of war’ between the apical polarity and actomyosin dynamics organize Kibra at the cell cortex to regulate Hippo signaling and tissue growth.

145 **The Centralspindlin proteins Pavarotti and Tumbleweed work with Wash to regulate Nuclear Envelope budding**
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The transportation of macromolecules between the nucleus and cytoplasm is indispensable in most developmental events such as differentiation, cell division, and aging. Nuclear envelope (NE-) budding is an alternate nuclear export pathway for macromolecules, which is analogous to the nuclear egress of herpesviruses. While NE budding is widely conserved from yeast to tissue culture cells, only a few players that regulate NE budding processes have been identified, including dFrizzled, Lamin, the Wiskott-Aldrich Syndrome protein Wash, the WASH regulatory complex (SHRC), and Arp2/3. Unexpectedly, we find Pavarotti (Pav) and Tumbleweed (Tum), which form the centralspindlin complex that regulates cytoplasmic actin and microtubules in many cellular processes such as cytokinesis, as new players in the NE budding process. Pav and Tum proteins localize in the nucleus, accumulate in NE buds, and their knockdowns exhibit

the loss of NE buds. To investigate the new roles of Pav and Tum in the nucleus, we examined how they function with known players during NE budding. We find that Pav and Tum can bind directly to Wash and make complexes in the nucleus. Interestingly, Tum makes a Pav-independent complex with Wash and SHRC, suggesting that Tum does not always work with Pav in the nucleus, unlike their canonical roles in the cytoplasmic. To further elucidate the mechanisms of NE bud formation by Wash, Pav, and Tum complexes, we generated a Wash mutant that cannot bind to Pav and Tum ($Wash^{\Delta P/T}$). $Wash^{\Delta P/T}$ exhibits the loss of NE bud phenotypes, indicating that Wash, Pav, and Tum complexes are essential for NE bud formation. We next examined the molecular function of $Wash^{\Delta P/T}$ during NE bud formation. Intriguingly, we find that $Wash^{\Delta P/T}$ loses actin bundling activity. To further elucidate actin bundling activity in the formation of NE buds, we examined the Pav^{DEAD} mutant that cannot bundle actin. Consistent with $Wash^{\Delta P/T}$, Pav^{DEAD} mutants exhibit the loss of NE bud phenotype. Thus, our results suggest that actin bundling by Wash and Pav in the nucleus has a vital role in the formation of NE buds. We are currently investigating how actin bundling by Wash and Pav regulates NE bud formation, as well as Tum function with Wash and SHRC during NE budding processes.

146 ***Drosophila* non-muscle myosin II Zipper positively regulates Notch signaling** Dipti Verma, Ankita Singh, Mousumi Mutsuddi, Ashim MukherjeeDepartment of Molecular and Human Genetics, Banaras Hindu University

The Notch pathway is an evolutionarily conserved signaling system which has been shown to play major role in cell fate determination, differentiation, proliferation and apoptotic events as well as self-renewal processes of different tissues. The same pathway can be deployed in numerous cellular contexts to play varied and critical roles for the development of an organism. The versatility of this pathway to influence different aspects of development comes from its multiple levels of regulation. In an effort to identify novel components involved in Notch signaling and its regulation, we carried out two independent protein-interaction screens, one based on the identification of cellular protein complexes using immunoprecipitation followed by mass spectrometry and other based on yeast two-hybrid system. Both the screens identified Zipper (Zip), also known as non-muscle myosin II (NM II), as an interacting partner of Notch. Physical interaction between Notch and Zip was further validated by co-immunoprecipitation studies. Immunocytochemical analyses revealed that Notch and Zip co-localise within the same cytoplasmic compartment. Different alleles of *zip* also showed strong genetic interactions with Notch pathway components in transheterozygous combinations. Downregulation of Zip resulted in wing phenotypes that were reminiscent of Notch loss-of-function phenotypes. The compromised activity of Notch pathway in absence of Zip was evident from the perturbed expression of Notch downstream targets, Cut and Deadpan. Significant accumulation of Notch receptor on the cell membrane was observed when Zip was abrogated. Further, synergistic interaction between these two genes resulted in highly ectopic expression of the Notch targets, Cut and Dpn. Both loss- and gain-of-function studies show that Zip positively regulates Notch signaling activity. Activated Notch-induced tumorous phenotype of larval tissues was enhanced by the overexpression of Zip. Co-expression of Zip and activated Notch together led to a hyperplastic wing phenotype with distorted morphology accompanied with loss of epithelial integrity and increased mitotic activity. Notch-Zip synergy resulted in the activation of JNK pathway that consequently lead to MMP activation and proliferation. Taken together, our results suggest that Zip may play an important role in regulation of Notch signaling.

147 **Natural variants in *SEL1L* modify lethality, ERAD, and proteasome function in a model of NGLY1 deficiency** Travis Tu'ifua, Clement Y ChowHuman 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 several disease phenotypes observed 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 known 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. Validating our screen, the *SEL1L*^{P780} and *SEL1L*$^{\Delta 806\text{-}809}$ variants increase the survival observed in the NGLY1 deficiency model, as compared to the more common *SEL1L*^{S780} variant. Further, we found that, as compared to *SEL1L*^{S780}, *SEL1L*^{P780} and *SEL1L*$^{\Delta 806\text{-}809}$ increase the NGLY1 model sensitivity to proteasome inhibition, a known defect in NGLY1 deficiency due to the misregulation of NRF1. We also find that these variants modify general ERAD function. We hypothesize that these *SEL1L* variants modify NGLY1 deficiency survival by reducing proteasome burden. 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.

148 **Modeling Glioblastoma angiogenesis in *Drosophila*** Anna Konstantinou, Ioanna Darioti, Thalia Moulka, Chrysoula PitsouliBiological Sciences, University of Cyprus

Glioblastoma multiforme (GBM) is the most malignant cancer type of the central nervous system and is characterized as highly angiogenic. Here, we used *Drosophila* to understand the molecular mechanisms underlying GBM tracheogenesis, the functional equivalent of human GBM angiogenesis. Using the Gal4-UAS system, we overexpressed the *Ras*^{V12}oncogene and the constitutively active catalytic subunit of PI3K, *Dp110*^{CAAX}, alone or in combination, in larval glia. We found that the mitotic index and size of brains overexpressing *Ras*^{V12} alone or in combination with *Dp110*^{CAAX} were strikingly increased compared to controls, whereas brains overexpressing *Dp110*^{CAAX} alone were moderately larger. In addition, brains overexpressing *Ras*^{V12} alone exhibited significantly increased apoptosis, a phenotype reminiscent of aggressive GBM. To understand glia-trachea interactions in GBM-*Ras*^{V12} larval brains, we developed two genetic models. In the first model, the *btl-mRFPmoesin* fluorescent reporter is used to visualize the cerebral tracheal arborizations and *repo-Gal4* drives expression of oncogenic *Ras*^{V12} and any other transgene in glia. In the second model, the QF-QUAS system is used to overexpress *Ras*^{V12} in glia and the Gal4-UAS system allows visualization and manipulation of the cerebral trachea. Both models behaved similarly with regards to GBM mitosis, growth and apoptosis. Importantly, our 3D imaging suggested increased tracheogenesis in brains overexpressing *Ras*^{V12} in glia in both models. Strikingly, downregulation of the FGFR/*btl* in tracheal cells of GBM-*Ras*^{V12} brains significantly decreased tracheogenesis, mitotic activity and tumor size underscoring the trophic role of tracheae in GBM growth. Ongoing experiments address the tissue-specific role of the Hif-1a/*Sima*-FGFR/*btl*-FGF/*bnl* pathway in GBM growth and tracheogenesis. These studies will establish *Drosophila* as a model for brain tumor tracheogenesis and provide novel insights for GBM angiogenesis.

149 **Elucidating the role of Tripod-Easel kinase complex in Wnt/Wg signaling** Muhammad Taha[1], Hassina Benchabane[1], Christin Carol Anthony[2], Leif Neitzel[2], David Robbins[3], Ethan Lee[2], Yashi Ahmed[11]Dartmouth, [2]Vanderbilt University, [3]Georgetown university

Every year 100,000 new cases of colorectal cancer (CRC) are diagnosed in US, which makes CRC the third leading cause of cancer-related deaths in the country. Nearly all CRC cases are caused by aberrant activation of the evolutionary conserved Wnt signaling pathway. Wnt signaling plays an important role in cell proliferation and differentiation during metazoan development. Stimulation of the Wnt pathway disrupts the activity of the destruction complex that targets the degradation of β-Catenin, the key transcription factor in the Wnt pathway. Consequently, β-Catenin accumulates in cytoplasm and enters the nucleus to activate Wnt target genes. Using a kinase overexpression screen in Xenopus embryos, we identified an evolutionarily conserved ser/thr Kinase (Tripod) as a positive regulator of the Wnt pathway. Building on this study, we investigated the role of an evolutionarily conserved binding partner and activator of Tripod, Easel. To determine whether Easel promotes Wnt signaling in physiological contexts, we performed loss-of-function studies in *Drosophila.* Our initial studies indicate that Easel, like Tripod, is a positive regulator of the Wnt signaling pathway. RNAi mediated knockdown of Scaffold in the wing disc caused loss of expression of a Wnt pathway target gene. Similarly, inhibition of Scaffold function resulted in notches in the adult wing and reduction of sternal bristles in the adult abdomen, both hallmarks of Wnt pathway inactivation. Moreover, mutations in Easel and Pygopus (a Wnt pathway transcriptional activator) resulted in deregulation of intestinal stem cell proliferation, another phenotype associated with Wnt pathway inactivation, revealing a genetic interaction between Easel and Wnt pathway components. These results show in vivo evidence about Easels role as an important regulator of Wnt signaling pathway.

150 **Efficacy of single domain antibodies targeting pathological tau protein in *Drosophila*** Sudershana Nair[1], Isabella Marchal[1], Hyung Don Ryoo[2], Einar Sigurdsson[11]Neuroscience and Physiology, New York University School of Medicine, [2]Cell Biology, New York University School of Medicine

Tau immunotherapies are promising approaches to treat Alzheimer's disease and other tauopathies. We have previously

reported by a transgenic approach that an anti-tau single chain variable antibody fragment (scFv) can clear pathological tau and suppress tau-associated neurotoxicity and lethality in *Drosophila* (Krishnaswamy et al. 2020). This finding supports future gene therapy approaches to target pathological tau aggregates. Single domain antibodies (sdAbs, 15 kDa) are more suitable for gene therapy than scFvs (30 kDa) because of their single unit, which facilitates proper folding within the cell and enhances solubility. To develop sdAbs for tauopathies, a llama was immunized with the longest isoform of recombinant tau protein (441 amino acids) and boosted with pathological tau isolated from human brain. Over 50 positive clones were identified via a phage display library derived from plasma polymorphonuclear cells that were isolated from high titer bleeds of the llama. Following characterization of their binding to various tau preparations, several were selected to examine their efficacy in culture and in vivo mouse and fly tauopathy models. For the fly studies, we generated five different sdAb transgenic flies, including one expressing control sdAb, and co-expressed them with UAS-tau^{R406W} flies only in neurons to determine their efficacy in clearing pathological tau, and preventing its neurotoxicity and lethality. We found that three of the anti-tau sdAbs were efficacious on these parameters, whereas the control sdAb had no effect. Although numerous tau antibodies, including sdAbs, have shown therapeutic promise in various models, the focus has been on their neuronal efficacy. It is unclear how well they work against glial tauopathies. We are examining this in fly models by expressing tau^{R406W} and the anti-tau sdAbs in glia. Our preliminary findings indicate that the two most efficacious sdAbs in neurons are also effective in glia in suppressing tau-associated lethality. These findings indicate their suitability as potential gene therapies for neuronal and glial tauopathies.

151 **Glycosphingolipid accumulation causes synaptic dysfunction in the *Drosophila* lipid storage disease model of Niemann Pick Type C** Anna E Eberwein, Kendal BroadieDepartment of Biological Sciences, Vanderbilt University

The lipid storage disease (LSD) Niemann Pick Type C (NPC) results in progressive childhood neurodegeneration owing to loss of the NPC1 (95%) and NPC2 (5%) genes. Lipids accumulated in NPC include cholesterol, sphingosine and glycosphingolipids (GSL). However, the key pathogenic lipids causing synaptic dysfunction and neurodegeneration are still unknown. We propose two linked hypotheses for the NPC disease condition; 1) GSL pathway mistrafficking of glucosylceramide (GlcCer) causes functional neurotransmission defects, and 2) this synaptic dysfunction underlies neurodegeneration. We are testing these hypotheses using an established *Drosophila* NPC disease model, with experiments focused at the well-characterized glutamatergic neuromuscular junction (NMJ) model synapse. We use two-electrode voltage-clamp (TEVC) electrophysiology to test changes in synaptic transmission. We have found that null *npc1a* mutants exhibit increased synaptic transmission, which is phenocopied in *brainiac* (*brn)* mutants, the GSL synthesis beta-1,3-N-acetylglucosaminyltransferase that causes mannosyl glucosyl ceramide (MacCer) accumulation. Importantly, double *brn; npc1a* null mutants have no additional increase in synaptic transmission, indicating that the *npc1a* and *brn* gene products operate in the same functional pathway. In addition, *egghead (egh)*, the GSL biosynthetic enzyme that glycosylates GlcCer, and *npc1a* double nulls have a similar increase in synaptic transmission to *npc1a* nulls, suggesting that MacCer accumulation is not responsible for the increase in synaptic transmission in NPC disease. Instead, Miglustat, a drug that inhibits GlcCer synthase, rescues the *npc1a* increase in synaptic neurotransmission, indicating that GlcCer accumulation is responsible for the change in synaptic transmission. To test synaptic dysfunction causal roles in NPC neurodegeneration, terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) has been used to measure cell death. We have found that *npc1a* null brains have elevated TUNEL labeling indicative of progressive neurodegeneration. We have been testing *brn* null mutants *and brn;npc1a* double nulls as well as *egh* and *egh;npc1a* double nulls with TUNEL labeling, as well as rescue by application of Miglustat to determine causal roles of GlcCer accumulation related to neurodegeneration progression. Taken together, these studies test GlcCer functions as a key pathogenic lipid in NPC synaptic dysfunction causally linked to progressive neurodegeneration.

152 **Modulation of purine biosynthesis via *Paics* suppresses disease phenotypes across multiple *Drosophila* models of Amyotrophic Lateral Sclerosis** Helen Y Zhou, Mathieu Bartoletti, Yuliya Nemtsova, Jonah F Boardman, Vikas A Rana, Camilla N Regalia, Kristi A WhartonDepartment of Molecular Biology, Cell Biology and Biochemistry and Carney Institute for Brain Science, Brown University

Amyotrophic lateral sclerosis (ALS), a neurodegenerative disease (ND) characterized by motor dysfunction, is associated with mutations in numerous genes including *SOD1*, *TARDBP*, *FUS*, and *C9ORF72*. Disruptions in homeostatic processes, including metabolism and autophagy, are common across all forms of ALS, indicating that restoration of cellular energy homeostasis may ameliorate disease progression regardless of etiology. Furthermore, increased autophagy, resulting from upregulation of glucose metabolism and nicotinamide cofactor synthesis, is neuroprotective in the context of ALS; however, the mechanistic basis of such a reversal of neurodegeneration is not understood.

We developed a conditional *Drosophila* model of *C9ORF72-(G4C2)49* ALS, wherein adult flies display a shortened lifespan. In a novel deficiency screen of the *Drosophila* X chromosome using this model, we found knockdown of *PAICS bifunctional enzyme* (*Paics*) suppresses *(G4C2)49*-associated neurodegeneration. *Paics* encodes a carboxylase-synthase enzyme in the *de novo* purine nucleotide biosynthesis pathway. We demonstrated that *Paics* knockdown also alleviates neurodegenerative phenotypes associated with *Drosophila TDP43*, *FUS*, and *SOD1* ALS models. Cell-type-specific knockdown of *Paics* in different components of the motor circuitry (glutamatergic neurons or cholinergic neurons) each rescue our *dSod1-G85R* knock-in model, corroborating previous work by our lab showing that non-motor neuron dysfunction is a major component of *SOD1* ALS disease progression. Investigations into the cellular correlates of rescue showed elevation of the autophagosomal membrane protein LC3/Atg8, indicating that *Paics*-mediated rescue of *dSod1-G85R* in both the glutamatergic and cholinergic neurons is accompanied by increased autophagy. We have also examined how *Paics* as a purine biosynthesis enzyme fits within the metabolic landscape of ALS by testing a number of genes acting in pathways both up- and downstream of *Paics*. Modulations diverting flux away from nucleotide biosynthesis rescued models of *C9ORF72*, *TDP43*, *FUS*, and *SOD1* ALS in a similar manner as *Paics*, highlighting how crosstalk between interconnected metabolic pathways can mediate ALS neurodegeneration. Overall, *Paics* knockdown both influences flux through adjacent metabolic pathways and increases autophagosomal activity to reverse ALS-associated energy imbalances.

In the context of previous work linking glucose metabolism and autophagy, our results suggest that downregulating purine biosynthesis may reestablish energy homeostasis in ALS by directing metabolites toward catabolic (e.g. glycolysis) rather than anabolic (e.g. purine synthesis) processes and thereby upregulating autophagy. Moreover, *Paics*-mediated rescue across different forms of ALS suggests a shared pathogenic mechanism of metabolic dysregulation that underlies ALS as a whole.

153 **N-acetylcysteine alleviates retinal defects in Drosophila models of *SNRNP200*-associated Retinitis Pigmentosa** Sara K Mayer[1], Quinton H Christensen[1], Hailey McCoy-Munger[1], Arlene V Drack[2], Lori L Wallrath[11]Biochemistry and Molecular Biology, University of Iowa, [2]Ophthamology, University of Iowa

Retinitis pigmentosa (RP) is a collection of genetic retinal degeneration disorders that affects 1:4000 individuals worldwide. RP is characterized by progressive photoreceptor cell death starting with rods and progressing to cones. Cell death occurs via apoptosis and the retina shows damage indicative of oxidate stress. RP33 is a non-syndromic form of autosomal dominant RP caused by mutations in the *SNRNP200* gene, which encodes a globally expressed pre-mRNA splicing factor possessing RNA helicase activity. To better understand how mutations in *SNRNP200* alter retina function, we developed Drosophila models of *SNRNP200*-associated RP. Drosophila possesses an orthologue of human *SNRNP200* which we call *dSnrnp200*. Human and Drosophila SNRNP200 exhibit 74% amino acid identity and 89% similarity. The objective of our studies is to understand how mutant *SNRNP200* causes retinal defects and identify potential treatments. To accomplish this goal, we introduced patient mutations into Drosophila *dSnrnp200* using CRISPR mutagenesis. These mutant *dSnrnp200* alleles cause a single amino acid substitutions in the active RNase helicase cassette. Similar to the human disease, mutations in *dSnrnp200* cause retinal defects and no additional overt phenotypes. The *dSnrnp200* mutants exhibit increased apoptosis in larval eye imaginal discs and an atypical arrangement of photoreceptors in the adult eye. Consistent with these abnormalities, electroretinograms show altered phototransduction in photoreceptors R1-6.

To determine the effects of depletion of *dSnrnp200* in the eye, RNAi was used to knock-down *dSnrnp200* in the developing eye. Depletion of *dSnrnp200* increases apoptosis in eye imaginal discs and produces a “rough eye” phenotype in adults. Given the connection between retinal cell death and oxidative damage, we treated flies depleted for *dSnrnp200* with N-acetylcysteine (NAC). NAC is an FDA approved antioxidant that showed cone improvement in a mouse model of RP and is currently being used in Phase I clinical trials to treat humans with RP. NAC treatment decreased apoptosis in the eye imaginal discs and showed dosage-dependent suppression of the rough eye phenotype in adults. Taken together, Drosophila models of *SNRNP200*-associated RP recapitulate aspects of the human disease, provided novel insights on disease mechanisms, and support antioxidants as a treatment for *SNRNP200*-associated RP.

154 **Time-restricted feeding retains muscle function through activation of purine cycles and AMPK signaling in Drosophila obesity models** Christopher Livelo[1], Yiming Guo[1], Farah Abou Daya[1], Vasanthi Rajasekaran[1], Shweta Varshney[2], Hiep Le[2], Stephen Barnes[1], Panda Satchidananda[2], Girish Melkani[11]University of Alabama at Birmingham, [2]Salk Institute for Biological Studies

Obesity caused by genetic predisposition, a lifestyle of calorie-dense diets, and circadian disruption can result in skeletal muscle dysfunction. Time-restricted feeding (TRF), where daily feeding was limited to only the active phase or first 12 hours during the day, led to improved skeletal muscle function compared to *ad libitum* feeding (ALF) counterparts in a high-fat diet (HFD)-induced and a genetic-induced obesity model (a *sphingosine kinase 2* mutant) using *Drosophila.* We evaluated a potential mechanistic basis of TRF-mediated benefits in muscle by utilizing temporal transcriptomic data of indirect flight muscle (IFM) followed by genetic validations using functional, cytological, biochemical, and metabolite assessment. Transcriptomic data for both obese models showed that TRF commonly upregulated genes involved in glycine production (*Sardh* and *CG5955*) and utilization (*Gnmt*), as well as downregulated a key gene (*Dgat2*) involved in triglyceride synthesis. IFM-specific knockdown (KD) of *Gnmt, Sardh,* and *CG5955* led to skeletal muscle dysfunction, ectopic lipid accumulation, and loss of TRF-mediated benefits. Further, IFM-specific KD of *Dgat2* retained muscle function during aging and reduced ectopic lipid accumulation, which mimicked TRF-mediated benefits. Interestingly, we found upregulation of genes and increases in metabolites related to the purine cycle predominantly under HFD-TRF. Furthermore, upregulation of genes and increases in metabolites associated with AMPK signaling, glycogen metabolism, glycolysis, TCA, and ETC were predominantly found in *Sk2*-TRF. Both AMPK signaling and the purine cycle led to increased levels of ATP in obese models under TRF conditions, indicating that ATP plays a potential role in TRF's ability to mediate muscle improvement. Overall, our data suggested that TRF improves muscle function through modulations of glycine production/utilization and triglyceride synthesis under obesogenic challenges. Further, distinct pathways namely the purine cycle and AMPK signaling were regulated under TRF in different obesity models and may contribute to potential mechanistic foundations that underlie TRF-mediated improvement in muscle.

155 **A single-cell transcriptomic study of Drosophila gastrulation highlights sequential transcription programs during mesodermal epithelial-to-mesenchymal transition** Jingjing Sun, Fan Gao, Chen Zhang, Angelike StathopoulosCalifornia Institute of Technology

Gastrulation is a crucial stage during development at which metazoan animals acquire cellular diversity and set up basic body plans. In this study, we used single-cell technology to investigate the dynamic transcriptional changes in the blastula and gastrula in order to provide insights into the molecular programs that drive the extensive cell movements and cell shape change associated with this important developmental stage. First, our results uncovered that two phases of gene expression are associated with the cellular blastoderm (nuclear cycle 14) or what is typically referred to as the major wave of zygotic gene activation. We also found that new progenitor cells are established at the posterior ventral region of the blastoderm that give rise to Malpighian tubules and a novel migratory cell population with similar transcriptional signature to founders of visceral muscles. Next, a differential gene expression (DGE) analysis identified a group of transcription factors, which previously only had been linked to ectodermal segment polarity, are expressed in the primordial mesoderm and required for its proper patterning and ultimately the differentiation of mesoderm cell lineages. These data suggest that the epithelial-to-mesenchymal transition (EMT), through which mesoderm cells acquire their migratory ability, is tightly coupled with the concomitant movement of the ectoderm. In summary, our work demonstrates that several sequentially-acting transcription programs are associated with gene expression changes in the early embryo even within a single developmental stage, gastrulation. These stage-specific changes were only illuminated through assay of gene expression with single-cell resolution. Furthermore, by highlighting these transcriptional activities both in time and in space, this study contributes to our understanding of how animals control and coordinate morphogenetic processes through rapid changes in developmental gene expression programs.

156 **Divergent transcriptional changes of *even-skipped* due to decreased *Krüppel* dosage** Shufan Lin[1], Bomyi Lim[21]Bioengineering, University of Pennsylvania, [2]Chemical and Biomolecular Engineering, University of Pennsylvania

Most organisms are viable as long as the genome carries one wildtype copy of the gene. Indeed, *Krüppel* (*Kr*[1]/+) heterozygous *Drosophila* are fertile and stable, even if the fly produces half the amount of Kr protein than wildtype. Nevertheless, the mechanism of maintaining the viability under heterozygous conditions is unclear. Is the reduced amount of Kr protein not sufficient to affect the downstream target gene expression? On the other hand, do downstream genes compensate for or tolerate this heterozygosity? Through quantitative live imaging utilizing the MS2/MCP and PP7/PCP system, we demonstrate that a Kr target gene *even-skipped* (*eve*) exhibits differential transcriptional dynamics to *Kr* dosage. Kr directly binds to the *eve* stripe 2 and 5 enhancers, and hence, *Kr* heterozygous embryos show a transient posterior expansion of the stripe 2 and an anterior shift of the entire stripe 5. Surprisingly, most significant

changes on *eve* stripes are seen in stripe 3 and 4, whose expression is not directly regulated by Kr. The mRNA production of stripe 3 is lower and the posterior boundary shrinks in Kr^{1}/+ embryos. The most affected stripe 4 displays an anterior shift of the entire stripe, delayed activation and reduced mRNA production. Our further investigation reveals that many gap genes including *giant* (*gt*) and *knirps* (*kni*) display modified gene expression kinetics in *Kr* heterozygous embryos. Halved Kr protein results in insufficient repression of these gap genes, inducing changes in their spatiotemporal patterns. As a result, both the posterior *gt* and *kni* shift anteriorly, while *gt* shifts earlier than *kni*. Such changes in gap genes coincide with the variation in *eve* stripe 3 and 4 dynamics. We propose that decreased *Kr* dosage affects a wide range of *eve* stripes through the combination of direct and indirect effects. Our study indicates that the spatial and temporal patterning of early embryos are sensitive to fluctuations in transcription factor concentration, resulting in more variability in the system.

157 **Odd-paired and Ocelliless dynamics in *Drosophila* head development** Shiva Abbasi[1], Kelli Fenelon[1], Fan Gao[2], Theodora Koromila[11]University of Texas at Arlington, [2]Caltech

Neural progenitor specification and differentiation occur early in development with detectable neuroblasts arising a mere one hour post gastrulation in the *Drosophila* embryo. The roles of transcriptional activators, such as Odd-paired (Opa)/ZIC3 and Ocelliless (Oc)/OTX2, are predominantly evolutionarily conserved. Opa is a late-acting pioneer factor that drives the transcriptional landscape to undergo a dramatic shift to prepare the syncytial nuclei for cellularization and transitioning the embryo into gastrulation. Also, a previous study showed that a group of Anterior-Posterior axis enhancers are initially activated by Bcd, and later activation is transferred to Oc via a feed-forward relay. In addition, gene replacement experiments show that the *Drosophila* Oc and orthologous mammalian Otx2 are functionally equivalent in the developing head. *oc* expression begins marginally before *opa* in the future head domain, but its role during cellularization is largely unexplored. Our hypothesis is that Oc and/or Opa are both regulating gene expression in the embryonic head, but only Oc is driving brain cell specification in the gastrulated embryo. Super-resolution, high-speed 4D imaging of both fixed and living embryos helped us quantify the overlapping expression regions before and after gastrulation in the *Drosophila* embryo. Single-embryo RNA-seq data reveals a subgroup of Opa-bound genes to be Opa-independent. Interrogation of these genes against pioneer factor ChIP-seq datasets and expression databases suggests that Opa acts together with Oc to regulate a subgroup of genes at cellularization. Additionally, we find that Oc supports gene regulation after gastrulation commencement by binding to brain-specific, Opa-independent enhancers during a third wave of zygotic transcription. Furthermore, Hi-M oligopaint technology allowed us to simultaneously study Oc and Opa expression profiles and chromosome organization in early neuroblasts. This research has clear potential to significantly advance the fields of epigenetics and developmental biology while elucidating the mystery of early embryonic brain specification. Funding: STARs program UTA

158 **Coordination of gene expression programs critical to development: chromatin modifier KDM5 in the prothoracic gland and beyond** Michael Rogers[1], Coralie Drelon[2], Owen Marshall[3], Julie Secombe[41]Genetics, Albert Einstein College of Medicine, [2]Albert Einstein College of Medicine, [3]Menzies Institute for Medical Research, University of Tasmania, [4]Genetics, Neuroscience, Albert Einstein College of Medicine

Proper development relies on coordinated gene expression programs to establish and maintain the functions of each cell type. Within this context, chromatin-regulating proteins act as powerful supervisors of the genome that are critical for the precise control of transcription. However, there remains much to discover about the mechanisms underlying this fine-tuning aspect of gene expression. Here, we expand our understanding of transcriptional dynamics by examining the developmental roles of the highly conserved histone modifier lysine demethylase 5 (KDM5, formerly Lid). *kdm5* is an essential gene in *Drosophila*, as null mutant animals die before eclosion, but interestingly, mutants lacking the canonical KDM5 demethylase enzymatic activity are viable as adults. These mutant models present a valuable opportunity to investigate demethylase-independent functions of KDM5 about which not much is known. Although KDM5 is ubiquitously expressed across the developing fly, the neuroendocrine prothoracic gland is a site of critical KDM5 functions for development and viability. In prothoracic gland cells, KDM5 doesn't directly regulate Halloween gene expression but rather plays critical roles in the cellular signaling networks upstream of ecdysone hormone biosynthesis. For proper developmental progression and metamorphosis, KDM5 functions in transcriptionally modulating MAPK signaling and the downstream promotion of endoreplication important for ecdysone production. In this study, we leverage Targeted DamID technology to assay KDM5 genomic targets and integrate these data with transcriptome, interactome, and cellular analyses to characterize KDM5-mediated programs of gene expression regulation. With this, the first genomic-binding data set in the prothoracic gland, we have been able identify both tissue specific cellular processes subject to KDM5-mediated transcriptional regulation as well as targets conserved across cell types and species.

Taken together, our studies provide key insights into the chromatin regulation critical to prothoracic gland function as well as expand our understanding of the functions of the transcriptional regulators like KDM5 that orchestrate development.

159 **Regulation of the *Drosophila* germ granules by granule mRNAs and proteins** Tatjana Trcek[1], Harrison A. Curnutte[2], Xinyue Lan[2], Manuel Sargen[2], Si Man Ao Ieong[2], Dylan Campbell[2], Sarah Bailah Lazar[21]Biology, Johns Hopkins University, [2]Johns Hopkins University

Germ granules are RNA granules that control mRNA abundance, translation and decay to specify the germ cell fate across species. Based on their morphology and biophysical properties, they resemble other RNA granules including processing bodies and stress granules, which are thought to degrade mRNAs or protect them during cellular stress, respectively. The *Drosophila* germ granules are nucleated by Oskar protein within a specialized cytoplasm termed pole plasm at the embryo's posterior. Removal of germ granules and the surrounding germ plasm abolishes germ cell formation, underscoring the observation that these structures are required for the fertility of the fly. However, apart from a few core germ granule proteins, little is known about how the components, resident to germ granules, instruct the formation of germ granules. In this report, we characterized the role of mRNAs and proteins in the formation of germ granules in *Drosophila*. Super-resolution microscopy revealed that the number, size and distribution of germ granules is precisely controlled. Contrary to other RNA granule, the formation and the persistence of germ granules does not rely on RNA itself but instead controls their size and composition. Using an RNAi screen, we determined that RNA regulators, helicases, and mitochondrial proteins regulate germ granule number and size while the proteins of the endoplasmic reticulum (ER), nuclear pore complex (NPC) and cytoskeleton control their anchoring and distribution. Therefore, the protein-driven formation of the *Drosophila* germ granules is mechanistically distinct from the RNA-dependent condensation observed for other RNA granules such as stress granules and P-bodies. Importantly, association of germ granules with the ER and NPCs carries important functional consequences for the development of germ cells. It could promote translation of germ granule mRNAs that code for signaling proteins and transcriptional regulators on the ER and NPCs to enable cell-to-cell communication and gene expression regulation and facilitate the establishment of the germ cell lineage. Research reported in this publication was supported by the NICHD R00HD088675 grant awarded to TT.

160 **Defining the transcriptional enhancers and regulators of *flamenco*, a prominent *Drosophila* piRNA cluster essential for female fertility.** Austin Rivera, Linda Yang, Jasmine Pierre, William Dorst, Rohit Sharma, Raghuveera Goel, Joseph Zaia, Nelson C LauBiochemistry, Boston University Chobanian & Avedisian School of Medicine

The *flamenco (flam)* locus is a prominent piRNA cluster expressed very highly in the *Drosophila* ovarian follicle cells with a known role to silence the gypsy transposons. There are two known *P-element*-induced mutations in the *flam* promoter region that cause loss of *flam* piRNAs and female infertility, but the overall promoter and enhancer architecture is poorly understood. To dissect the transcriptional regulation of *flam,* we engineered new promoter bashing constructs to evaluate the transcriptional activity of the *flam* piRNA cluster promoter element. Contrary to an earlier report suggesting transcriptional regulation from a minimal promoter element upstream of the *flam* transcriptional start site, our study suggests that the entire first exon of *flam* is the most potent enhancers of *flam* expression.

To test the impact of these new putative enhancers, we used CRISPR/Cas9 editing to create new deletion mutants in the *flam* enhancer regions. We determined a significant reduction but not complete loss of transcription from *flam*, and then assayed the mutants for egg development and fecundity assays. Consistent with the diminished but not complete loss of *flam* transcription, these enhancer mutants mostly had atrophied ovaries and no progeny.

To discover the protein transcription factors (TFs) that bind these new putative *flam* enhancers, we applied quantitative proteomics on pulled-down DNA constructs incubated with *Drosophila* Ovarian Somatic Sheet (OSS) cell whole lysate. Our top TF candidates from this proteomics screen were *biniou*, a forkhead domain TF; *apontic*, a a DNA-binding protein that acts as a transcriptional regulator; and *traffic jam*, the large Maf transcription factor critical for male and female gonadogenesis. We will report at the meeting our validation of these candidate factors in functional assays in OSS cells as well as transgenic fly systems.

161 **An undergraduate-driven bioinformatics screen reveals novel factors that target the *Drosophila melanogaster* histone gene locus** Lauren J Hodkinson, Connor Smith, Casey A Schmidt, Leila E RiederBiology, 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 initiated a bioinformatics candidate-based screen as part of a course-based undergraduate research experience (CURE), where students mapped publicly available ChIP-seq datasets to the histone gene array. Using this approach, we confirmed the expected localization of the general transcription factor TRF2, and found histone promoter targeting by other general transcription factors including TAF1, TFIIB and TFIIF. We also identified novel transcription factor HLB candidates, including the Hox proteins Ultrabithorax, Abdominal-A, and Abdominal-B, suggesting a link between body plan morphogenesis and histone production. In addition, we discovered that a kinase (JIL-1), a hormone receptor-like protein (Hr78), and the long isoform of a BET-family protein (fs(1)h L) exhibit histone array localization. Our current work includes wet-lab experiments to follow up on these bioinformatics hits. This screen provides several candidates for future studies into factors that influence histone biogenesis. Further, our approach emphasizes the powerful reservoir of publicly available datasets, which can be mined as a primary screening technique for finding factors that target any genomic locus of interest.

162 **Generating enhancer variability and testing activity in a high-throughput manner** Julia Falo Sanjuan, Arman Karshenas, Claudia Medrano, Alex Haugan, Joey McKenna, Meghan Turner, Jenna Haines, Hernan Garcia, Mike EisenUniversity of California Berkeley

Understanding the rules that govern gene expression remains a major unsolved challenge in biology. Regulatory sequences, termed enhancers, are composed of binding sites for transcription factors, whose number, affinity, spacing and orientation can play a role in enhancer function. Although the roles of some of these factors are well established, the rules by which binding site arrangement dictate gene expression programs remains poorly understood. We could predict enhancer function if we could test all functional and non-functional enhancer sequences (the 'enhancer space'), but testing 4^500 variants (for a 500bp enhancer for example) is not a feasible option. To circumvent this, we are generating tools to generate high a degree of enhancer sequence variability, in silico by learning from evolutionary variants and generating new ones and in vivo using base editing. We are also optimizing assays to test the activity of all these variants in a high throughput manner, using STARR sequencing in transgenic S2 cells and in Drosophila embryos by single cell RNA sequencing. We show mutations can be introduced in vivo using the base editor AID fused to nCas9, and generation of transgenic S2 lines that express different combinations of transcription factors to test enhancer activity in cell culture.

163 **TFTag – A novel library of endogenously tagged *Drosophila* transcription factors** Korneel Hens, Sebastian KittelmannCentre for Functional Genomics, Oxford Brookes University

Differential gene expression is central to most biological processes in multicellular organisms and is largely regulated at the level of transcription. Transcription factors (TFs) bind to *cis*-regulatory elements in the DNA and enhance or repress activity of RNA polymerase II. The combined action of all TFs per gene largely determines the respective expression output but the causative molecular mechanisms remain elusive for many TFs. There are 753 known or predicted sequence-specific DNA-binding TFs annotated in the *Drosophila* genome and the roles of about 275 have been characterised to at least some extent. However, this knowledge is usually limited to one or only a few tissues, and relatively little is known about the remaining 478.

To understand the function of a TF, it is necessary to study its tissue distribution, binding characteristics at physiological concentration, effect on transcription, and protein interactions. Unfortunately, the combination of tools for such a comprehensive study is rarely available. To address this, we have recently developed the Exchangeable Tagging System (ExTaSy), which enables the introduction of a tag into the endogenous gene locus using CRISPR/Cas9. This primary tag can be exchanged for any other tag making the system highly versatile. Moreover, the use of a transgenic marker that can be excised with piggyBac transposase allows for virtually scarless modification of the locus. Using ExTaSy, we have started to generate a community resource, TFTag, to enable the in-depth study of TF function in *Drosophila*. This resource consists of three parts: a set of plasmids for the tagging of all *Drosophila* TFs, fly lines in which TFs have been tagged, and a database with expression and binding information for a subset of previously unstudied TFs. Importantly, individual tagging constructs are cloned for each annotated protein terminus, allowing for analysis of different TF variants and reducing the risk of possible interference of the tag with TF function. As a proof of principle, we have tagged different

variants of Ovo/Shavenbaby, Ocelliless/Orthodenticle, and Tramtrack and have started to analyse their expression and binding in different contexts. Our results will provide important insights into TF function and transcriptional regulation, and our TFTag library will be a useful and highly versatile resource for a wide range of *Drosophila* researchers.

164 **Using single-cell RNA sequencing to generate cell-type-specific split-GAL4 reagents throughout development** Yu-Chieh David Chen[1], Yen-Chung Chen[1], Raghuvanshi Rajesh[1], Nathalie Shoji[1], Maisha Jacy[1], Haluk Lacin[2], Ted Erclik[3], Claude Desplan[11]New York University, [2]Washington University in St. Louis, [3]University of Toronto

The proper function of the nervous system relies on the recognition of different neurons to form circuits. Understanding how functional neuronal circuits are built requires the generation of cell-type-specific tools during development. *Drosophila* has emerged to be a powerful genetic model organism for studying neuronal circuit formation due to the development of sophisticated genetic intersectional techniques such as the split-GAL4 system that allows single-cell resolution. While many existing genetic tools enable highly cell-type-specific labeling in the adult brain, they often fail to show expression during development. For example, neurotransmitters or their receptors are often used as genetic markers for labeling a subset of cell types, but they are not expressed early when circuits form. Here, we use single-cell RNA sequencing (scRNAseq) data in the developing *Drosophila* visual system as a model for generating highly cell-type-specific genetic split-GAL4 lines labeling neurons throughout development.

Our lab recently produced a single-cell transcriptomic atlas for all optic lobe neurons at different stages of development of the *Drosophila*visual system and identified around 250 distinct cell types. We used these datasets to develop a systematic pipeline for analyzing gene expression in each cell type across various developmental stages. We identified pairs of genes that are expressed together in only one or a few clusters and generated split-GAL4 drivers that restrict expression to cells where the two genes of the split-GAL4 regulator are co-expressed. These split-GAL4 lines can be generated through the Recombinase-mediated cassette exchange (RMCE) of T2A-split-GAL4 elements from the large existing collection of MiMIC/CRIMIC lines, or by C-terminal knock-in of T2A-split-GAL4 elements through CRISPR genome editing. We find extremely high accuracy in labeling predicted cell types using these split-GAL4 lines whose expression was consistent from early developmental stages to adulthood. This collection of highly cell-type-specific developmental split-GAL4 lines also facilitates the annotation of unidentified clusters in our scRNAseq dataset and the identification of novel cell types. Finally, the gene-specific split-GAL4 toolkit developed in our study is adaptable to other systems in *Drosophila*. Therefore, the scRNAseq-guided split-GAL4 strategy provides highly cell-type-specific developmental genetic tools to study key genes controlling various neuronal developmental processes.

165 **Cas9-derived Nickase promotes efficient allelic conversion through Homologous chromosome-Templated Repair (HTR) in somatic tissues** Annabel Guichard[1], Sitara Roy[2], Sara San Juste[2], Ankush Auradkar[2], Zhiqian Li[2], Ethan Bier[21]UCSD, [2]Cell and Developmental Biology, UCSD

Repair of double-strand breaks (DSBs) in somatic cells is primarily achieved by error-prone nonhomologous end joining (NHEJ) and, less frequently, by precise Homology-Directed Repair (HDR), which preferentially employs the sister chromatid as template. Here, we present a system designed to reveal repair using intact sequences from the homologous chromosome following Cas9-induced DSBs at the *white* locus through the production of red (*white*$^{+}$) clones in otherwise white (*white*$^{-}$) eyes. We find that this process, referred to as Homologous chromosome Templated Repair (HTR), performs at frequencies of 20-30%, competing with higher rates of NHEJ (~70%). Unexpectedly, HTR was much more efficient (40-65%) in response to single-strand breaks (SSBs) induced by the nickase variants of Cas9 Cas9D10A or Cas9H840A. Repair phenotypes elicited by Nickase versus Cas9 differ in both developmental timing (late versus early stages, respectively) and the production of undesired mutagenic events (rare versus frequent). Importantly, we demonstrate that chromosome pairing is not strictly required for HTR, and that sequence homology alone can sustain SSBs-induced HTR. We perform an internally controlled RNAi screen to identify DNA repair factors implicated in HTR. Such Nickase-mediated HTR represents an efficient and unanticipated mechanism for allelic correction, with far-reaching potential applications in the field of gene editing.

166 **Physical aspects of Drosophila gastrulation** Konstantin Doubrovinski[1], Mohamad Ibrahim Cheikh[2], Miriam Osterfield[31]Biophysics, UT Southwestern Medical Center, [2]Biophysics, [3]UT Southwestern Medical Center

Drosophila gastrulation is a popular model used to study morphogenesis. Despite a long-standing effort to determine the physical nature of cell shape changes in this key model system, there is no consensus on the underlying biophysical mechanism. Any predictive model of a morphogenetic event requires the knowledge of material properties of the tissue undergoing morphogenesis. Using our previously developed methods to apply pulling force to a single cell of an early

embryo, we were able to quantify the profile of tissue deformation and the dynamics of tissue recoil after the force is released. Comprehensive computational modeling suggest that these data can only be explained assuming that apical domains are much softer than both the lateral and the basal domains. Motivated by this prediction, we developed a novel protocol to probe individual cellular domains using iron microspheres. Strikingly, applying concentrated pulling force to apical and lateral cellular domains resulted in formation of remarkably long membrane tethers. Tether formation required orders of magnitude smaller force than our typical global tissue deformations when the force probe contacts the basal side. Our measurements thus suggest that cells are extremely rigid on their basal side, whereas lateral and basal domains are orders of magnitude softer, likely lacking stable membrane-associated cytoskeleton. Furthermore, using the newly developed AID-2 degron, we show that (nearly) complete depletion of myosin II has no effect on the mechanical measurements, indicating that the measured responses are passive. A novel 3D computational model integrating our experimental findings suggests that (1) cell elongation during the early phase of gastrulation is a passive process driven primarily by viscous shear forces, and (2) tissue invagination must require active tension in the lateral membranes as well as forces transmitted through the cytoplasm.

167 **A second-generation auxin-inducible gene expression system for conditional manipulation of *Drosophila* circadian behavior** Khushbakht F Butt[1], Sergio L Crespo-Flores[1], Seanna Kelly[1], Colin McClure[2], Amira Hassan[3], Tony D Southall[3], Annika F Barber[1,11]Rutgers, the State University of New Jersey, [2]Queen's University Belfast, [3]Imperial College London

The ability to control transgene expression, both spatially and temporally, is essential for studying model organisms. In *Drosophila*, spatial control is primarily provided by the GAL4/UAS system, whilst temporal control relies on a temperature-sensitive GAL80 (which inhibits GAL4) and drug-inducible systems. However, these are not ideal. Shifting temperature can impact on many physiological and behavioural traits, and the current drug-inducible systems are either leaky, toxic, incompatible with existing GAL4-driver lines, or do not generate effective levels of expression. AGES relies on the auxin-dependent degradation of a ubiquitously expressed GAL80, and is therefore compatible with existing GAL4-driver lines. Here we describe a second-generation Auxin-inducible Gene Expression System (AGES2). Water-soluble auxin analog is added to fly food at a low, non-lethal, concentration, which induces gene expression comparable to uninhibited GAL4 expression. AGES2 improves upon the original system by implementing a bump-and-hole mutation strategy that renders the system sensitive to the synthetic ligand 5-Phe-IAA. AGES2 offers three major improvements: 1) reduced leakiness of expression; 2) lower required ligand concentration to avoid potential side-effects; 3) more rapid onset of gene expression. AGES2-induced expression of fluorescent markers is robust in both larvae and adults across multiple tissues. AGES2-induced manipulation of the excitability of clock and sleep circuitry using overexpression, RNAi, or CRISPR approaches rapidly and robustly alters behavior, with no effect of 5-Phe-IAA feeding on the behavior of control flies. The AGES2 system offers a stringent, non-lethal, cost-effective, and convenient method for temporally controlling GAL4 activity in *Drosophila* and is suitable for behavioral control.

168 **A new arena to measure humidity preference in wild type and mutant *Drosophila* species** Daniel T Bennett[1], Genevieve Jouandet[1], Matthew Capek[2], Marco Gallio[1], Alessia Para[11]Neurobiology, Northwestern University, [2]NUIN, Northwestern University

Environmental humidity influences the fitness and geographic distribution of all animals. Insects in particular use humidity cues to navigate the environment, both to avoid the danger of desiccation and to find suitable sources of water for egg laying and other activities. Yet, the molecular and cellular basis of humidity sensing remains poorly understood. We and others have recently described genes and neurons necessary for hygrosensation in the common fruit fly *Drosophila melanogaster*. We also described divergent humidity preference in *D. melanogaster*, the afrotropical species *Drosophila teissieri* (native to humid rainforests in west equatorial Africa), and the nearctic *Drosophila mojavensis* (native to the dry desert of the Southwestern United States and Mexico). Our results demonstrated that humidity preference is both innate and species specific. As a starting point towards a deeper understanding of the genetic mechanisms underlying the evolution of humidity preference in *Drosophila*, our goal has been to develop robust and reliable assays to measure hygrosensory responses in *Drosophila melanogaster* and other species, and use this assay to compare behavior in wild type and mutants for key hygrosensory genes. Here, we present a new assay specifically designed to measure humidity preference in *Drosophila* melanogaster and other *Drosophila* species. We will describe its design and present preliminary results of its application.

169 **Automated cobot-assisted high-throughput phenotyping of cognition in behaving *Drosophila melanogaster*** Riddha Manna[1], Tomislav Štampar[2], Ivan Tomić[1], Ana Marija Jakšić[11]School of Life Sciences, École

Polytechnique Fédérale de Lausanne, [2]Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb

Cognition is the ability to acquire information from an external environment, retain it as knowledge, and apply it to adaptive behaviors through information processing. *Drosophila melanogaster*, exhibits all four core components of cognition (perception, memory, learning, and decision-making), making them an excellent model to study cognition on a behavioral as well as neuronal and molecular level. To score fly cognition effectively, we need to track the flies' behavior and performance in a cognitively challenging task and use it as a proxy for cognition due to our inability to effectively communicate with them. This is a challenging task for us as well because complex behaviors tend to exhibit high intra-as well as inter-individual variability. Thus, many individual measurements in a high number of flies are needed to attain the statistical power necessary for differentiating cognitive performance from chance-events. However, most *Drosophila* studies report their findings based on group behavior or a small number of individuals. Few efforts have been made to study cognitive behaviors in individual flies with sufficiently high throughput.

To fill this gap, we developed a flexible, automated, high-throughput and closed-loop system that allows us to dynamically present the fly with visual stimuli in the form of colored patterns displayed on an LCD coupled with electric shock reinforcement. As we will demonstrate here, this system enables us to score various associative cognitive tasks in 64 individual flies at a time in a Y-shape closed-loop maze, including but not limited to classical and operant conditioning, (delayed)(non-) matching to sample tasks and visual perception. The shape of the maze and the experimental design is only limited by a space constraint of 30 x 30 mm^2 and the researcher›s imagination. Further, the rig›s design is modular and flexible for use with collaborative robotic arms. In a robotic platform we developed the robot is able to manipulate single flies and set up the full 64-fly assay with no human intervention. Using continuous robotic assistance in this experiment potentially increases the throughput to phenotype 1500 flies per day. We have successfully validated our system by scoring wild-type and memory-mutant flies in a place-learning operant conditioning assay while the maximum throughput will be demonstrated by scoring variation in learning in individual flies across 200 wild-type genotypes.

170 **New technology and resource development at the Drosophila Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR) and DRSC/TRiP** Justin Bosch[1], Matthew Butnaru[1,2], Aram Comjean[1], Ben Ewen-Campen[1], Elizabeth Filine[1], Corey Forman[1], Srishti Goswami[1], Claire Yanhui Hu[1], Ah-Ram Kim[1], Grace Kim[1], Raphael Lopes[1], Enzo Mameli[1], Stephanie E Mohr[1], Kelly Reap[1,2], Alexandria Risbeck[1], Emily Stoneburner[1], Raghuvir Viswanatha[1], Baolong Xia[1], Jonathan Zirin[1], Norbert Perrimon[1,21]Harvard Medical School, [2]Howard Hughes Medical Institute

The NIH NIGMS-funded Drosophila Research and Screening Center-Biomedical Technology Research Resource (DRSC-BTRR; NIH P41 GM132087) serves as a technology development, optimization, and dissemination platform for the *Drosophila* and broader research community. As the DRSC-BTRR, the current iteration of the DRSC and TRiP, our group focuses on the development and optimization of CRISPR and other technologies in partnership with collaborating labs, as well as the dissemination of mature technologies. One area of focus for our group is on pooled-format CRISPR knockout screens in *Drosophila* and mosquito cell lines. Through optimization of our genome-wide sgRNA library and CRISPR/Cas strategies, including through use of an anti-CRISPR protein, we have further improved this powerful method for large-scale investigation of cellular gene function. We are further innovating through testing of new CRISPR/Cas strategies in *Drosophila* cells and *in vivo*, and through development of new online resources for reagent design, data mining, and data integration. Here, we present an overview of newly available technologies, resources, and online tools, as well as examples of how they can be efficiently and effectively applied as components of small- and large-scale biological and biomedical research studies.

171 **On being the right size: the nuclear-to-cytoplasmic ratio control of early embryogenesis** Yuki A Shindo, Anusha Bhatt, Madeleine Brown, Kiera Schwarz, Shruthi Balachandra, Amanda A AmodeoBiology, Dartmouth College

Cells of a given cell type must maintain a typical cell size for proper physiological functioning. Cell size control requires that division be balanced by growth so that the correct size is maintained. My lab is interested in how cells measure their size to maintain this balance. We have focused primarily on the early cleavage divisions of the Drosophila embryo as a model system where the relationship between size and cell cycle progression is particularly evident. The newly fertilized egg is an unusually large cell. To reduce cell size and increase cell number, the first hours of Drosophila development are dominated by a series of rapid, reductive, syncytial nuclear divisions. These divisions lack both growth phases and bulk of zygotic transcription and therefore are driven by maternally provided products. Once a sufficient number of nuclei are made the cell cycle pauses to allow cellularization of the syncytium during a developmental transition known as the mid-blastula transition (MBT). The final cell size is determined by the number of nuclei that the embryo has generated

by cellularization. Strikingly, the ratio of nuclei to cytoplasm (N/C ratio), which directly correlates to final cell size, determines when the cell cycle stops and cellularization occurs. My lab explores the molecular mechanisms by which the N/C ratio is sensed. We have identified histones, particularly histones of the H3 family, as critical players in this process. We explore the ways in which the changing N/C ratio affects nuclear and chromatin composition, production of specific zygotic products, and cell cycle regulation to uncover the principles of cell size control employed by the early embryo.

172 **Unraveling the Neural Circuitry of Female Aggression in Health and Disease** Caroline Palavicino-MaggioHarvard Medical School

How aggression is orchestrated remains one of the most fundamental questions of neurobiology, with crucial implications in understanding circuit development and disease. While aggression in males of many species has been studied dating back to the 1900s, female aggression studies have been largely ignored. Our work set to solve a major and still-unanswered question in the field: how is female aggression encoded in the brain? Using a genetic approach and a large library of fly lines we used a behavioral screen to identify a single line, that targeted a specific population of neurons. We identified a female-specific subgroup of cells in the pC1 brain region, known as pC1α neurons. These pC1α neurons when activated through a heat sensitive cation channel triggered female flies to fight at high intensity levels and no change in male behavior suggesting that aggression is sexually dimorphic. In other words, aggression is wired differently in males than in females, representing an exciting new paradigm in the field. These findings offered insight into a longstanding question of the underlying female aggression circuitry and laid the foundation for a new field delineating female-specific aggression circuitry as well as candidate aggression genes.

173 **Genome instability in stem and progenitor cells** Allison BardinInstitut Curie

Mutations arising in adult stem cells often lead to the initiation of pre-cancerous lesions providing a selective fitness advantage. Our recent work in *Drosophila* and that of others in mammalian model systems have demonstrated that adult stem cell mutation is frequent and can have significant phenotypic consequences on adult tissues. Importantly, the underlying mutational processes remain to be fully understood. I will present our recent data using whole-genome Illumina and Nanopore sequencing approaches to characterize somatic mutations arising in adult stem cells. In addition, I will discuss unpublished work on the effects of induced replication stress in adult stem cells and how this may be buffered in some tissues. Overall, our findings highlight how replication stress and genome instability affect developing and adult tissues.

174 **Going in circles gets you somewhere – signaling mechanisms that coordinate cell movements for rotational epithelial migration** Sally Horne-BadovinacUniv Chicago

Collective migration of epithelial cells plays central roles in the morphogenesis, homeostasis, and pathogenesis of tissues. For epithelial cells to migrate collectively, the cytoskeletal machinery that powers each cell's movement must be polarized across the tissue plane so that the entire epithelium can move in a directed way. In a wound healing scenario, the empty space at the wound edge provides an external cue to polarize the tissue for directed movement. However, epithelia that adopt circular or spherical geometries can spontaneously migrate in either a clockwise or counterclockwise direction in the absence of a polarizing cue. In these rotational epithelial migrations, the cells rely entirely on local cell-cell interactions to self-organize for collective movement. How this occurs is unknown. In this talk, I will discuss how we have used the rotational migration of follicular epithelial cells in the Drosophila egg chamber to begin to unravel the molecular logic underlying this self-organization process.

175 **Encoding Memory in Amyloids** Kausik SiStowers Institute for Medical Research

Memory is a feature of biological systems. In its simplest form, memory is a systems' ability to change its function in response to a specific stimulus and maintain that altered state even after the stimuli cease to exist. Our work in nervous system illustrates that regulatable amyloids, which relies on a protein's ability to adopt multiple conformations, have affordances relevant to biological memory: switch-like feature, self-sustaining change in function, and a stable yet malleable state.

Often amyloids are studied outside of their biological context, although a key feature of many amyloid-based diseases is context-specificity. Studying amyloids in cellular context, as part of normal biological processes, as opposed to pathological anomalies, we hope to understand several unresolved questions: e.g., why through evolution have proteins

retained the ability to form amyloids if they are inherently toxic? and why do widely expressed, multifunctional proteins, lead to tissue or cell -type specific diseases? Importantly, the discovery that cells can not only assemble, but also rapidly disassemble/dissolve amyloids as part of their normal biology, provides a path to discover hitherto unknown cellular mechanisms to control amyloids.

176T **PINK1 in *Drosophila* Bcl-2 family protein dependent apoptosis** Mélanie Fages[1], Aurore Rincheval[1], Sylvina Bouleau[1], Isabelle Guénal[21]Univesity Paris Saclay, UVSQ, LGBC, [2]Université Paris Saclay, UVSQ, LGBC

Apoptosis is programmed cell death, allowing physiologic elimination of cells during development as well as damaged cells in adult metazoans. This sophisticated process involves mitochondrial membrane events implicating, among others, many actors of mitochondrial dynamics (fusion, fission) or mitochondrial quality control (mitophagy). Many links exist between these two processes and the apoptotic machinery. Some members of the Bcl-2 (B cell Lymphoma 2) family, identified as apoptosis actors, can modulate mitochondrial fission and fusion. Moreover, mitophagy regulators such as PINK1 (PTEN Induces Kinase 1) can modify some Bcl-2 family protein activity and, in this way, prevent apoptotic pathways.

On the other side PINK1 mutation is associated with several types of genetic or sporadic forms of Parkinson's disease. Consequently, PINK1 appears to be a protective protein regarding cell death. By contrast, our results in the *Drosophila* model suggest a positive role for PINK1 in apoptosis induced by *Rbf1* (the retinoblastoma susceptibility gene homolog). These results reinforced the idea of a close link between apoptosis and mitophagy. This poster presents our first results of the PINK1 role in the Bcl-2 family protein-dependent mitochondrial apoptosis.

177T **Endogenous Retroviruses and TDP-43 Proteinopathy Form a Sustaining Feedback to Drive the Intercellular Spread of Neurodegeneration** Yung-HENG CHANG[1], josh dubnau[21]Anesthesiology, Stony Brook School of Medicine, [2]Anesthesiology/Neurobiology and Behavior, Stony Brook School of Medicine

Neurodegenerative disorders are thought to initiate focally and then spread over time through neural circuits. A mechanism that has been proposed to explain this is the inter-cellular movement of misfolded "prion-like" proteins that then recruit normally folded proteins in recipient cells to also adopt pathological conformations. Such a mechanism has been proposed, for example, to explain the propagation of abnormally phosphorylated cytoplasmic inclusions of TAR-DNA-Binding protein (TDP-43) during the progression of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)4. But unlike transmissible prion diseases such as mad cow disease, which can be caused by ingestion or by injection into animal brains of misfolded PrP, ALS and FTD are not infectious and the injection of phosphorylated and aggregated TDP-43 protein into rodent brains is not sufficient to cause disease phenotypes unless those animals also express high levels of transgenic TDP-43 protein. This suggests a missing component of a positive feedback that is necessary to sustain disease progression. We provide evidence that endogenous retroviruses (ERVs) are that missing component. We demonstrate that ERV expression and TDP- 43 proteinopathy are mutually reinforcing. Expression of either the Drosophila ERV, gypsy (mdg4) or the human ERV, HERV-K (HML-2) are each sufficient to stimulate cytoplasmic aggregation of human TDP-43. Importantly. We also demonstrate that viral ERV transmission causes propagation of such TDP-43 pathology to cells that express physiological levels of TDP-43, whether they are in contact or at a distance. This mechanism underlies the toxicity of glial cells to neurons and other glia in a Drosophila in vivo model.

178T **Irradiation-induced cell migration is regulated by caspases executed through EMT and triggered by the cytosolic DNA sensing pathway** Lena Sapozhnikov, Rela Oved, Eli AramaMolecular Genetics, Weizmann Institute of Science

The last two decades have seen a rapid expansion in the number of discoveries and studies investigating different paradigms of caspase-dependent non-lethal cellular processes (CDPs). We previously discovered that applying ionizing radiation to *Drosophila* larvae which are severely compromised for caspase activity, leads to massive transition of the "undead" epithelial cells of the imaginal discs to highly migratory and invasive cells[1]. We showed that this phenomenon, which we termed irradiation-induced cell migration (ICM), is potently attenuated by low levels of effector caspase activity, far below the threshold required to induce apoptosis. In addition, ICM can also occur normally during development in caspase compromised epithelial tissues, presumably by the developmentally "undead" cells. However, it remained unclear how the epithelial cells acquire migratory and invasive capacities. Furthermore, the signaling pathway which triggers ICM, as well as the possible entry points for regulation by caspases, remained elusive.

Here I will present our unpublished data demonstrating that ICM is driven by the epithelial-mesenchymal transition

(EMT) process. I will also provide evidence that ICM is triggered by the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling pathway, which recently has been emerged as a key DNA-sensing machinery in innate immunity. Finally, I will highlight several key components of the EMT process and the cGAS-STING pathway that are potential caspase substrates and are also critical for ICM, offering several entry points for regulation by caspases.

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179T **The role of metabolites in Minute cell physiology and cell competition.** Alex Mastrogiannopoulos[1], Remi Logeay[1], Michael Baumgartner[1,2], Paul Langton[1], Eugenia Piddini[11]School of Cellular and Molecular Medicine, University of Bristol, [2]The Perelman School of Medicine at the University of Pennsylvania

Mutations in ribosome protein (Rp) or *Minute* genes and in ribosome biogenesis factors result in debilitating diseases known as ribosomopathies. Cells heterozygous mutant for Rp genes (Rp/+) also get eliminated by cell competition when mixed with wild-type cells. The competitive elimination of *Minute* cells is thought to act as a quality control mechanism to eliminate subfit cells and is also likely relevant to cancer. Recent studies in *Drosophila* have focused on how Rp/+ cells experience stress pathway activation and transcriptional changes as an adaptation to single copy loss of Rp genes. Such transcriptional profiles reveal that Rp/+ cells have differential expression of several metabolic enzymes. Our recent work finds that some of these enzymes act as modulators of *Minute* cell competition and that the corresponding metabolites show altered levels in *Minute* cells. This suggests that metabolites can act as mediators or modulators of Minute cell competition.

180T **Wdr59 promotes or inhibits TORC1 activity depending on cellular context** Yingbiao Zhang[1], Chun-Yuan Ting[2], Shu Yang[2], Lucia Bettedi[2], Kuikwon Kim[2], Mary Lilly[21]NICHD, NIH, [2]NIH/NICHD

Target of Rapamycin Complex I (TORC1) is a central regulator of metabolism in eukaryotes that responds to a wide array of negative and positive inputs. The GATOR signaling pathway acts upstream of TORC1 and is comprised of two subcomplexes. The trimeric GATOR1 complex inhibits TORC1 activity in response to amino acid limitation by serving as a GTPase activating protein (GAP) for the TORC1 activator RagA/B, a component of the lysosomally located Rag GTPase. The multi-protein GATOR2 complex inhibits the activity of GATOR1 and thus promotes TORC1 activation. Here we report that Wdr59, originally assigned to the GATOR2 complex based on studies performed in tissue culture cells, unexpectedly has a dual function in TORC1 regulation in *Drosophila*. We find that in the ovary and the eye imaginal disc brain complex, Wdr59 inhibits TORC1 activity by opposing the GATOR2 dependent inhibition of GATOR1. Conversely, in the *Drosophila* fat body, Wdr59 promotes the accumulation of the GATOR2 component Mio and is required for TORC1 activation. Similarly, in mammalian HeLa cells, Wdr59 prevents the proteolytic destruction of GATOR2 proteins Mio and Wdr24. Consistent with the reduced levels of the TORC1 activating GATOR2 complex, Wdr59KOs HeLa cells have reduced TORC1 activity which is restored along with GATOR2 protein levels upon proteasome inhibition. Taken together, our data support the model that the Wdr59 component of the GATOR2 complex functions to promote or inhibit TORC1 activity depending on cellular context.

181T **Exploring the effects of heat shock stress on nucleolar structure and function** Anna S Ramsey, Danielle E Talbot, Bailey J Vormezeele, Tina TootleAnatomy and Cell Biology, University of Iowa

The nucleolus is a membraneless organelle that plays a critical role in cell signaling and has many cellular functions, including ribosome biogenesis, organizing heterochromatin, regulating the cell cycle, and activating the cellular stress response. The structure and function of the nucleolus are deeply connected. The nucleolus is a phase separated organelle, meaning the interactions between functional nucleolar components drive the formation and thus, the structure of the nucleolus. Using *Drosophila* oogenesis, our lab found inhibition of RNA polymerase I (RNAPI) and loss of prostaglandin synthesis distinctly alter nucleolar function and structure. Therefore, we wanted to explore what changes in nucleolar function and structure can be caused by other stimuli. Here we focus on the effects of heat shock. We find that heat shock does not result in a significant change in nucleolar morphology observed by GFP-Fibrillarin. This result was consistent with either a single heat shock (37 °C for 5, 10, 30, or 60 minutes) and repeated heat shock-recovery cycles (37 °C for 3 30-minute heat shock cycles with 15- or 30-minute recovery periods). Although we did not see alterations to nucleolar structure, we observed decreased GFP-Fibrillarin levels in heat shocked nucleoli. Because changes in nucleolar function precede changes in nucleolar structure, we wanted to see if nucleolar function was altered by the heat shock conditions. To do this, we used a nucleotide incorporation assay to label newly synthesized rRNA. Heat shock results in decreased rRNA, suggesting that rRNA transcription is decreased by heat shock. These data indicate that

the nucleolus may respond differently to acute and chronic stimuli. During short periods of heat shock stress, the cell may downregulate transcription in favor of maintaining critical cellular functions for after the stress is resolved. Whereas chronic stress will both inhibit transcription and drive changes in nuclear structure. Understanding the effects of different stimuli like heat shock on the nucleolus can provide greater insight into cellular mechanisms of how cells cope with short term environmental stressors compared to stresses stemming from underlying diseases, such as cancer.

182T **Investigation of mechanisms underlying cell corpse clearance in the brain of glia-specific phagocytosis-deficient flies** Guangmei Liu, Johnny Elguero, Kim McCallBoston University

Billions of cells die per day to maintain homeostasis during development, growth and aging. In healthy condition, dead cells are rapidly engulfed and degraded by phagocytes through phagocytosis. Draper (Drpr) is a crucial phagocytic receptor on the surface of phagocytes to recognize dead cells in *Drosophila*. Our lab previously found that knocking down *drpr* in glia lead to accumulation of neuronal corpses over time, actively dying glia in the brain, and age-dependent neurodegeneration. These neuronal corpses are neurons that died during larval and early pupal development. 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 how glial cell corpses are cleared. In addition, we have found that knockdown of Relish suppresses the neurodegeneration seen in aged *draper* mutants, and we are investigating potential targets of Relish that mediate this suppression. Progress towards these aims will be presented.

183T **Characterizing the Role of p38Kb and GARS in CMT** Piotr Klos[1], MacKenna Duncan[1], Lauren Naeger[1], Megan Cross[1], Julia Martin[1], Alysia Vrailas-Mortimer[1,21]Illinois State University, [2]Department of Biochemistry and Biophysics, Oregon State University

Charcot-Marie-Tooth Disease (CMT) is a progressive neuropathology caused by the deterioration of neuronal function of the peripheral motor and sensory nervous systems. Symptoms include tripping, ankle twisting, and clumsiness. Effects on the sensory system include sensations such as pins and needles and burning pain. There are no preventive therapeutics for CMT, and treatment revolves around managing symptoms. Though mutations in a variety of genes can give rise to CMT, several of the genes are tRNA-synthetases. We have recently found that the p38 MAPK (p38Kb), which regulates aging and age-dependent locomotor behaviors, regulates the levels of several tRNA-synthetase proteins during aging. Since p38Kb interacts with the Chaperone-Assisted Selective Autophagy (CASA) complex to mediate the degradation of misfolded or nonfunctional proteins, this suggests that during aging, certain tRNA-synthetase proteins become damaged and are normally cleared by p38Kb and the CASA complex. Failure to clear these damaged proteins potentially results in disease symptoms or worsening of symptoms. I hypothesize that p38Kb-mediated regulation of tRNA synthetase degradation is crucial for maintaining proper neuromuscular function. I will utilize D. melanogaster to test interactions between p38Kb and the tRNA synthetase GARS and how this contributes to CMT-like phenotypes in flies by determining if p38Kb can mediate the clearance of mutant GARS from the cell. Overexpression of p38Kb in muscles improves locomotor function, and newer evidence suggests that mutant GARS expression in the mesoderm is sufficient to induce neuropathology. Future research with p38Kb can help us better understand its role in progressive diseases.

KEYWORDS: Charcot-Marie-Tooth Disease; CASA complex; p38Kb; tRNA synthetases; Drosophila melanogaster; neuromuscular junction

184T **Developmental parthanatos of the primordial germ cells is regulated by lipid metabolism** Eli Arama, Guy Hadary, Lama Tarayrah-Ibraheim, Keren Yacobi SharonWeizmann Institute of Science

Parthanatos is an alternative form of cell death which is biochemically and molecularly distinct from apoptosis, autophagy, or necrosis, and has been implicated in the pathogenesis of several important human diseases, including Parkinson's disease, stroke, heart attack, diabetes, and ischemia reperfusion injury in numerous tissues. Although parthanatos has been almost exclusively investigated under non-physiological conditions in mammalian cells, we recently reported the discovery of a developmental form of parthanatos, through which about 30% of theprimordial germ cells (PGCs) are normally eliminated during early *Drosophila* embryogenesis[1]. Since genetic manipulations of apoptosis performed almost two decades ago by several groups, have all failed to affect PGC death, the notion that this cell death pathway diverges from the conventional apoptotic program has emerged. In accordance, a few genes unrelated to apoptosis have been reported to affect PGC death, including two genes, (*wunen*) *wun* and *wun2*, encoding for lipid phosphate phosphatases (LPPs), and have been shown to act redundantly in the germ cells and the soma to regulate PGC

migration and cell death.

To explore the signaling pathways and execution mechanisms underlying PGC death by parthanatos, PGCs isolated during relevant embryonic stages were subjected to single-cell RNA sequencing (scRNAseq) and subsequent clustering analysis. Significantly, the expression patterns of several genes important for PGC biology and cell death were instrumental in identifying the most relevant cluster of the PGCs that are doomed to die, and consequently a few new genes were identified, including the *Drosophila Perilipin/ADRP* gene homolog, *lipid storage droplet 2* (*lsd2*). Given the crucial role of the Wunens in mediating PGC death and their molecular function as LPPs in the metabolism of lipid phosphates, we explored possible involvement of lipid metabolism pathways in PGC death. Here, I will present our most recent findings associating PGC death with lipid metabolism.

185T **The role of *Baldspot/Elovl6* in metabolic homeostasis upon dietary stress** Nicholas Molisani, Rebecca PaluBiological Sciences, Purdue University Fort Wayne

The endoplasmic reticulum (ER) stress response has been shown to play a pivotal role in the progression and outcome of many metabolic diseases. This occurs when misfolded proteins accumulate in the ER activating the unfolded protein response (UPR). This response tries to bring the organelle back to cell homeostasis for proper protein synthesis, but chronic activation of this response will lead to cell dysfunction and apoptosis. Understanding the molecular processes that regulate the UPR could give us a better understanding of how to treat these diseases. An ER-associated enzyme involved in the activation of the UPR, fatty acid elongase *ELOVL6*, is responsible for converting palmitate to stearate and reduced expression of *ELOVL6* has been shown to reduce cell degeneration and apoptosis in other models of ER stress involving metabolic disease. For this research, we will be investigating how expression of *Baldspot,* an ortholog to *ELOVL6* in *Drosophila melanogaster,* influences metabolic states in response to dietary stress. We believe that reducing expression of *Baldspot* in *D. melanogaster* will allow for better regulation of metabolic states by reducing the activation of the UPR when exposed to dietary stress. Preliminary results show that larvae with reduced expression of *Baldspot* on a high sugar diet aren't developmentally delayed. We also see that adult flies with reduced *Baldspot* expression have higher triglyceride concentrations (stored fats) on a high sugar diet compared to the controls. Ultimately, we believe that *Baldspot/ELOVL6* could be an excellent target in UPR-related diseases, including metabolic disease.

186F **Investigating the role of the TNF pathway in Drosophila tricellular junction protein regulation** Zazil Adriana Solis Saldivar[1], Vanessa Auld[21]Department of Cellular and Physiological Sciences, University of British Columbia, [2]Department of Zoology, University of British Columbia

Epithelial cells have the critical role of establishing and maintaining permeability barriers. These barriers are vital to maintaining different molecular/ion compositions for organ function, and their deregulation causes disruption of tissue homeostasis and even cancer. In Drosophila polarized epithelia, permeability barriers are formed between two cells by septate junctions and the corners, where three cells converge, by the tricellular junction (TCJ). TCJs are comprised of specific protein complexes that are tightly regulated in both mammals and Drosophila, yet little is known about the mechanisms that regulate them. Gliotactin is a key protein for TCJ development in Drosophila. When overexpressed in the wing imaginal disc, Gliotactin spreads away from the TCJs, leading to deleterious phenotypes such as apoptosis, over-proliferation, and cell migration. Overexpression of TCJ proteins in other systems leads to the same phenotypes suggesting that regulation of TCJ proteins is an essential feature of epithelial integrity. Our data shows that the cytokine tumor necrosis factor (TNF) signaling pathway enhances these phenotypes. This conserved pathway regulates cell survival and death by activating the JNK pathway. The TNF receptor Grindelwald mediates the pro-apoptotic functions of Eiger, the unique Drosophila TNF ligand. Downregulation of Grindelwald and downstream signaling components suppresses the overexpression phenotypes of Gliotactin. However, the downregulation of Eiger does not. The project's overall objective is to determine the function of the TNF signaling pathway in mediating Gliotactin overexpression phenotypes. Gliotactin is internalized when overexpressed through endocytosis, and our data shows that Grindelwald is internalized in the same endocytic vesicles (a key step for receptor activation). We hypothesize that overexpression of the TCJ protein, Gliotactin, activates the TNF signaling pathway through Grindelwald in a ligand-independent manner. We will test this hypothesis by quantifying the impact of Gliotactin overexpression in the wing disc in conjunction with manipulating the TNF pathway. This research could reveal a novel Grindelwald activation mechanism and establish a link between the TNF pathway, apoptosis, and TCJ protein misregulation in Drosophila.

187F **Higher hypoxia-sensitivity of pupae than embryos in *Drosophila melanogaster*** Tsering Stobdan, Nicholas J Wen, Ying Lu-Bo, Dan Zhou, Gabriel G HaddadUCSD

Hypoxia not only plays a critical role in multiple disease conditions but it also influences the growth and development of cells, tissues and organs. Earlier, we and others have shown that both embryo and pupae in *Drosophila melanogaster* are critical hypoxia-sensitive stage. While severe hypoxia, i.e., 2% O_2, elicits embryonic cell-cycle arrest and a relatively moderate hypoxia, 5% O_2, leads to death of approximately 80% of pupae, it is still ambiguous as to what stage in Drosophila development is more sensitive to hypoxia especially in relation to survival and adaptation. Since a precise identification of a hypoxia-sensitive developmental stage could also help identify critical genes involved in hypoxia, we systematically examined different stages of *Drosophila* development for its hypoxia sensitivity. We found that 4% O_2 exposure from embryo to larvae does not have much impact on *Drosophila* eclosion. Interestingly, the sensitivity increases with pupae stage, i.e., 99.6%, 66.1% and 54.4% eclosion rate when returned to normoxia at early, mid and late pupae stages, respectively. The pupae sensitivity is further substantiated by total lethality of normoxia-grown pupae when treated with 4% O_2 at any pupae stages i.e., early, mid and late pupae stage. To further characterize the specific pupa stage and critical time duration, pupae were subjected to 4% O_2 at different times in pupation and for different time durations. Exposing early pupae for 3 days of 4% O_2 led to only 6.1% eclosion, while shorter exposure such as for 2 days and 1 day to 4% O_2 resulted in 66.7% and 96.4% eclosion. We also performed the same experiments in our 'laboratory generated' hypoxia-adapted fly lines. Interestingly, despite being able to complete their life cycle in 4% O_2, we found that 4% O_2 was lethal for adapted flies when they were grown in normoxia condition and were treated to 4% O_2 only during the pupae stage. These results indicates that *Drosophila* pupae are most hypoxia-sensitive stage. Additionally, the results from adapted flies indicates the involvement of developmental selection or epigenetic mechanism that protect the flies from increased stress levels during metamorphosis.

188F **Molecular investigation of UQCRC1 in a disease model of neurodegeneration** Yu-Chien Hung[1], Kuan-Lin Huang[1], Jeng-Lin Li[2], Po-Lin Chen[3], Han-Yi Lin[2], Wen-Chun Lo[1], Shu-Yi Huang[4], Chun-Hong Chen[3], Chin-Hsien Lin[2], Chih-Chiang Chan[11]Graduate Institute of Physiology, National Taiwan University, [2]Department of Neurology, National Taiwan University Hospital, [3]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, [4]Department of Medical Research, National Taiwan University Hospital

Human Ubiquinol-Cytochrome C Reductase Core Protein 1 (UQCRC1) is an evolutionarily conserved core subunit of mitochondrial respiratory chain complex III. We recently identified the disease-associated variants of UQCRC1 from patients with familial Parkinsonism, while its function remains unclear. Flies with neuronal knockdown of *uqcrc1* exhibit age-dependent parkinsonism-resembling defects, including dopaminergic neuron reduction and locomotor decline, and are ameliorated by *UQCRC1* expression. Lethality of *uqcrc1*-KO is also rescued by neuronally expressing *UQCRC1* but not the disease-causing variant, providing a platform to discern the pathogenicity of this mutation. Furthermore, UQCRC1 is associated with the apoptosis-triggered cytochrome *c* (cyt-*c*)*,* and *uqcrc1* deficiency increases cyt-*c* in the cytoplasmic fraction and activates the caspase cascade. Depleting cyt-*c* or expression of the anti-apoptotic p35 ameliorates uqcrc1-mediated neurodegeneration. However, we showed that the regulation of the apoptosis pathway could partially rescue the phenotypes caused by loss of UQCRC1 and indicated that the other activated cellular mechanisms may be involved in the maintenance of neuronal survival together. The abnormal autophagy mechanism may be considered for assistance in neurodegeneration induced by loss of UQCRC1 in neurons.

189F **Assessment of *ACBP* and its impact in retinal degeneration** Landin Stokes[1], Maxwell Jenkins[2], Rebecca Palu[11]Biological Sciences, Purdue University Fort Wayne, [2]Biology, Indiana Tech

Apoptosis is an essential component in an organism's developmental process, but when misregulated it can lead to degeneration, or cancer. Variation in apoptosis is therefore critically linked to outcomes in these diseases. In a previous study, a model of apoptosis (overexpression of *rpr*) was crossed to the *Drosophila* Genetic Reference Panel (DGRP) to identify over 100 individual candidate modifier genes, including the acyl-CoA binding proteins. *ACPB* is associated with the binding of acyl-CoA ester outside the cellular membrane, for entry into the cell. Acyl-CoA is an important component in the Krebs cycle, which is an important energy production function. Without this interaction, an excessive amount of acyl-CoA esters accumulate in the cytoplasm, causing the mechanical activation of mitochondrial degradation, activation of caspase proteases, and eventually apoptosis. Using one of the three genes that are essential for activation of caspase pathways responsible for apoptotic activity, we assess the consequence of knock-down of this gene in degenerative model *Drosophila Melanogaster*. An earlier study utilized *rpr* to assess gene interactions with the *rpr* caspase pathway. The purpose of the study was to observe whether any given gene's interaction and their amplification or degeneration of apoptotic activities within a degenerative model. To determine if the acyl-CoA binding proteins impact degeneration, we used RNAi to reduce their expression in each of three models of retinal degeneration: overexpression of *rpr,* overexpression of *p53*, and overexpression of $Rh1^{G69D}$. We are also utilizing the TUNEL assay to verify that any changes

in degeneration are linked to changes in apoptosis. We have found that loss of *ACBP5* expression results in increased degeneration in all three apoptotic models. Our findings suggest that the loss of *ACBPs* greatly impacts cellular development and the cellular stress response.

190F **Identifying Proteins that Mediate Cellular Behaviors in Response to Higher Intracellular pH** Laura Martins, Ramy Wong, Daniel Orozco, Bree Grillo-HillBiology, San Jose State University

Proliferation is a key cellular process that is tightly regulated in cells and essential for proper growth of multicellular organisms. Many studies have identified genes and proteins that are essential for regulated proliferation, but much less is understood about environmental factors that control proliferation, such as intracellular pH (pHi). pHi is tightly regulated by cells, and emerging evidence suggests regulated pHi dynamics modulate regulated cell proliferation. To regulate pHi, cells use a wide variety of ion exchangers and acid loaders/extruders to maintain pH near physiological levels. NHE1 in mammals (DNhe2 in Drosophila) is an ubiquitously expressed sodium proton exchanger that acts as a rheostat to maintain physiological pH. In diseases such as cancer, cells have constitutively increased pHi which in turn alters functions of pH sensitive proteins leading to altered cell behaviors, like increased proliferation. However, it is unknown which specific pH sensitive proteins are dysregulated at an increased pHi. To study the role of dysregulated pHi in cancer, our lab generated transgenic flies that inducibly express DNhe2, the homolog of NHE1, in the Drosophila eye. In previous work, our lab demonstrated that overexpression of DNhe2 in developing Drosophila tissues is sufficient to increase pHi and increase cell proliferation in vivo, and results in a rough eye phenotype in adult flies. Here we describe a reverse genetic screen to identify candidate pH-sensitive proteins that promote cell proliferation. We screened a collection of 193 Drosophila lines covering 94% of the second chromosome. We visually inspected flies for enhancement or suppression of the GMR>DNhe2 rough eye phenotype. We identified 35 regions of the second chromosome that show an interaction with GMR>DNhe2. We are focusing on two overlapping deficiencies that both suppressed the GMR>DNhe2 rough eye phenotype, Df (2L)ED1303 and Df(2L)ED1315, spanning 38B4-38C6. We identified 8 candidate genes in the region defined by the overlapping deficiencies. We obtained genetic reagents to alter expression of each gene, and tested them for suppression of GMR>DNhe2. Two of these genes, CG10949 and CG31688, showed genetic interactions with predicted loss-of-function alleles. Next, we will quantify the effects of candidate genes by counting cells and assaying molecular markers in third larval instar eye imaginal discs. Understanding the effects of increased pHi and which possible pH sensitive proteins induce hyperproliferation can help us understand and possibly uncover therapeutic targets.

191F **Evidence for existence of an apoptosis-inducing BH3-only protein, *sayonara*, in *Drosophila*** YUko Ikegawa, Yoo Sa KanRIKEN

Cells need to sense stresses to initiate execution of the dormant cell death program. Since the discovery of the first BH3-only protein Bad in 1995, BH3-only proteins have been recognized as indispensable stress sensors, which induce apoptosis. It has been regarded over the last two decades that BH3-only proteins do not exist in *Drosophila* in spite of their importance in other organisms. Here we identify the first *Drosophila*BH3-only protein and name it *sayonara*. Sayonara induces apoptosis in a BH3 motif-dependent manner and interacts genetically and biochemically with the Bcl-2 homologous proteins, Buffy and Debcl. There is a feedback loop between Sayonara-mediated caspase activation and autophagy. The BH3 motif of *sayonara*phylogenetically appeared at the time of the ancestral gene duplication that led to the formation of *Buffy* and *Debcl* in the dipteran lineage. This is the first identification, to our knowledge, of a bona fide BH3-only protein in *Drosophila*, challenges the established concept in the field and provides a unique example of how cell death mechanisms can evolve both through time and across taxa.

192F **HATSDAL suppresses the endoplasmic reticulum stress response in high ambient temperature** Kim Il-Ju, Kim Il-JuSchool of Life Sciences, Gwangju Institute of Science and Technology

High ambient temperature has a significant impact on the growth and physiology of animals, especially poikilothermic organisms such as the fruit fly, *Drosophila melanogaster*. To survive in adversely high ambient temperatures fruit flies have developed a variety of behavioral and physiological adaptations. Nervous system-linked temperature sensors allow fruit flies to actively avoid unfavorably high ambient temperatures (28°C>). However, when forced to grow at 30°C, Drosophila larvae accelerate growth and reach adulthood in 7 days, whereas at 25°C, they develop in 9 days. This suggests that Drosophila larvae effectively cope with stresses associated with high ambient temperatures. To better understand how Drosophila larvae develop at temperatures above the optimum, we searched for mutants that develop normally 25°C but not at 30°C and name them 'high ambient temperature-sensitive developmental arrest

loci (HATSDAL)'. Here, we identified HATSDAL, a gene essential for Drosophila to complete larval development at 30°C. HATSDAL mRNA was expressed in the imaginal discs with higher expression at 30°C compared to 25°C. When incubated at 30°C, larvae deficient of HATSDAL showed altered endoplasmic reticulum (ER) stress genes, particularly those related to the protein kinase R-like ER kinase (Perk). Moreover, loss of function mutations in the Perk pathway rescue the developmental arrest of the HATSDAL1-deficient mutant at 30°C, suggesting a genetic link between HATSDAL and the Perk pathway. We currently are investigating the mechanism by which HATSDAL alters the ER stress response. We hope that this study will elucidate the genes and signaling processes that determine the threshold of the ER response.

193F **The G protein-coupled receptor kinase Gprk2 acts in follicle cells to regulate nurse cell death and corpse clearance via two pathways** Jeanne S. Peterson[1], Diane V. Lebo[2], Kimberly McCall[21]Biology, Boston Univ, [2]Biology, Boston University

In an RNAi screen for kinases affecting ovarian function, the knockdown of G-protein coupled receptor kinase 2 (*Gprk2)* in follicle cells was found to disrupt both the removal of nurse cell nuclei in late stages of oogenesis as well as the dumping of nurse cell cytoplasm into the oocyte. Since mutations of *GPRK2* are homozygous semi-lethal, knockdowns and mosaic analysis were used to characterize the phenotype. The relationships between *Gprk2* and the *Drosophila* homologs of the *C. elegans* engulfment pathways, *ced-1*/*-6*/*-7* and *ced-2*/*-5*/*-12,* were investigated and the results will be shown. We also investigated knockdowns of candidate interacting GPCR genes, *methuselahlike* -5 and -10 and these data will be presented. Our results indicate that GPRK2 acts in the Draper (Ced-1) pathway and a separate pathway that affects nurse cell dumping.

194F **Feedback circuit that simultaneously drives cell death and proliferation** Shivakshi Sulekh, Yuko Ikegawa, Sa Kan YooBDR, RIKEN

Caspases are traditionally known to execute apoptosis. In addition, roles for caspases in non-apoptotic processes have been noted, but their exact mechanisms remain elusive. Here, using *Drosophila* intestinal stem cells (ISCs), we discovered that weak activation of caspases induces massive cell proliferation rather than cell death. We reveal an existence of a feedback circuit between caspases and JNK, which simultaneously drives cell proliferation and cell death. This feedback loop exists in both ISCs and imaginal disc cells, but it is defective in ISCs due to inefficient DIAP1 regulation, leading to the caspase-induced proliferation in ISCs. This work provides a molecular mechanism by which caspases perform apoptotic and non-apoptotic functions *in vivo*.

195F **A cell death sensitivity switch in long-lived cells** Jessica Sawyer, Ruth A Montague, Don T FoxDepartment of Pharmacology & Cancer Biology, Duke University

Many metazoan tissues harbor long-lived cells that are not replenished by tissue regeneration. In the absence of regeneration, it is advantageous for long-lived cells to resist diverse cellular insults. However, the molecular underpinnings that enable cellular longevity within a tissue are still largely unclear. We have established the adult hindgut of *Drosophila melanogaster* as a model to understand responses to tissues injury and cell death in a long-lived organ with no cell turnover. The *Drosophila* hindgut is comprised of three distinct segments. At the anterior is the pylorus, which is a contractile organ that moves food along the alimentary canal. The ileum and rectum are important for salt and water balance. We have previously shown that ectopic expression of pro-apoptotic genes *hid* and/or *rpr* causes cell death and regenerative hypertrophy in the pylorus. Here, we show that expression of *hid* does not activate a caspase sensor or lead to cell death in the mature adult hindgut ileum or rectum. In contrast, the ileum and rectum are sensitive to ectopic expression of caspase *dronc* (Caspase-9), a downstream *hid* target. Unlike the regenerative response to cell death in the hindgut pylorus, cell death in the ileum does not activate a regenerative program. Our results suggest that insensitivity to the upstream cell death signal Hid underlies cellular longevity in the ileum. The *hid*-sensitive pylorus and *hid*-insensitive ileum are derived from the same progenitor population during metamorphosis. Therefore, we wanted to identify when this change in *hid*-sensitivity arises. We find that young animals (less than one day post eclosion) are *hid*-sensitive and mature animals (3-4 days post eclosion) are *hid*-insensitive. Our results suggest that long-lived, non-regenerative ileal cells lose sensitivity to cellular insults as the adult hindgut matures. Currently, we are conducting gene expression analysis to identify factors that are responsible for this cell death sensitivity switch. Our results demonstrate that the *Drosophila* hindgut is an excellent model to reveal unique molecular regulation underlying a tradeoff between regeneration and longevity.

196S **Mechanism of induction of a stabilizing protein in degenerating photoreceptors** Heena Khurana, Thi Le, Khanh Lam-Kamath, Deepshe Dewett, Jens RisterBiology, University of Massachusetts Boston

Rhodopsin 1 (Rh1, encoded by *ninaE*) is a seven transmembrane protein that localizes to the light-sensing compartments, called rhabdomeres, of the fly photoreceptors. Rh1 consists of an opsin protein that is covalently bound to the visual chromophore 11-*cis* retinal. In humans as well as in flies, 11-*cis* retinal is derived from vitamin A and is required for the synthesis of Rh1. Lack of vitamin A prevents the synthesis of the retinal chromophore and leads to the production of immature Rh1 that localizes to the cell body. Interestingly, the lack of mature Rh1 affects rhabdomere size, but does not cause photoreceptor death in the fly eye. This led us to hypothesize that certain molecules might be upregulated in response to vitamin A deficiency to stabilize the damaged photoreceptors.

Our lab showed that a novel transmembrane protein, Major Photoreceptor Stabilizer (Mps), stabilizes the damaged photoreceptors in vitamin A deprived flies. However, the mechanisms that trigger Mps expression are not understood. Since vitamin A deprived flies express immature Rh1, we examined if Mps responds to immature Rh1 irrespective of vitamin A deficiency. In this regard, we dissected $ninaE^{8}$ mutants that have missense mutation in gene encoding Rh1, resulting in abnormally processed Rh1. We found that, $ninaE^{8}$ mutants, like vitamin A deficient flies, also express immature Rh1 that mostly localizes to the cell body and exhibit smaller rhabdomeres. Mps was localized to the outer photoreceptors.

To examine Mps expression, we also took advantage of *Drosophila* model for retinitis pigmentosa, $ninaE^{G69D}$, that are known to process Rh1 differently in two different genetic backgrounds. Colley et al (1995) found that $ninaE^{G69D}/+$ flies (flies expressing one mutant copy of $ninaE^{G69D}$ over wild type copy) express no or low levels of immature Rh1 with faint levels of mature Rh1 as seen on the blots. However, immature Rh1 is expressed in $ninaE^{G69D}/-$ flies (expressing mutant $ninaE^{G69D}$ over the deletion of *ninaE* locus). Using immunohistochemistry, we showed that $ninaE^{G69D}/+$ flies express mature Rh1 that localizes to the outer photoreceptors and exhibit low levels of Mps. Interestingly, $ninaE^{G69D}/-$ mutants, similar to $ninaE^{8}$ mutants and vitamin A deprived flies, have smaller rhabdomeres and express immature Rh1 that localizes to the cell body (with some Rh1 localizing to the outer photoreceptors) and high levels of Mps in the outer photoreceptors. Further we knocked down *mps* to confirm if it stabilizes the damaged photoreceptors in $ninaE^{8}$ and $ninaE^{G69D}/-$ mutants. Strikingly, knockdown of *mps* in both these mutants completely damaged the rhabdomeres, causing a fused ring structure. Taken together, our data suggest that immature Rh1 triggers the expression of the transmembrane protein Mps that stabilizes damaged photoreceptors.

197S **An inducible Actin Stress Response disrupts the balance between pools of nuclear and cytoplasmic actin in embryos** Natalie Biel[1,2], Faizan Rashid[1], Anna M Sokac[1]1Cell and Developmental Biology, University of Illinois Urbana Champaign, [2]Integrative Molecular and Biomedical Sciences Program, Baylor College of Medicine

Environmental stress can negatively impact embryo development. Exposure to heat is an example of environmental stress that has teratogenic effects. However, how heat affects early embryos and their developmental processes is still poorly understood. We previously identified an inducible Actin Stress Response (ASR) in cellularizing *Drosophila* embryos that are exposed to mild heat stress (32°C). ASR is known to occur in other adult cell types following exposure to heat, oxidative stress, or the accumulation of aggregates of β-amyloid and is associated with myopathies and neurodegenerative disease. Our work marks the first report of an ASR in an embryo, and we also found it to be associated with reduced embryo survival. In embryos, the ASR is characterized by an inducible increase in activity of the F-actin depolymerizing protein Cofilin, a consequent destabilization of cytoplasmic filamentous actin (F-actin), and the formation of intranuclear actin rods. We propose a model where increased Cofilin activity drives destabilization of F-actin structures in the cytoplasm, increasing the global pool of free monomeric actin in the cytoplasm, and subsequently driving up the level of monomeric actin in the nucleus to feed assembly of actin rods. We further hypothesize that the increased influx of actin into nuclei during the ASR is detrimental to embryo health. Consistent with our model, we have found that the number of intranuclear actin rods is directly related to the free actin concentration in the nucleus. Therefore, we can use the number of actin rods as a readout for the level of actin in the nucleus. To further test the predictive power of our model, we are using genetic mutants to manipulate the levels and stability of F-actin in the cytoplasm and then measuring the number of actin rods in the nucleus. To better understand how the ASR impacts embryo health, we are also using RNASeq to assay the physiology of heat stressed embryos that can or cannot mount an ASR. Together, these results will further our understanding of the ASR, its implications for developing embryos, and will provide novel insight into the interplay between cytoplasmic and nuclear actin pools in cells, which is an understudied area of actin biology.

198S **The Role of Ca^{2+} Signaling in Apoptosis-induced Proliferation** KOMAL SUTHAR[1], Andreas Bergmann[21]Department of Molecular, Cell and Cancer Biology, UMASS MEDICAL SCHOOL, [2]Department of Molecular, Cell

and Cancer Biology, UMass Chan Medical School

Calcium (Ca^{2+}) plays a major role in many cell biological processes to maintain tissue homeostasis. Apoptosis-induced Proliferation (AiP) maintains tissue homeostasis in which apoptotic cells send signals to their surviving neighboring cells to induce their proliferation. To examine the role of Ca^{2+} signaling in AiP, we used the "undead" AiP model in larval eye imaginal discs in which simultaneous overexpression of the proapoptotic gene *hid* and the effector caspase inhibitor *p35* maintains the cells in an undead state. In this undead model, the NADPH oxidase DUOX generates extracellular reactive oxygen species (ROS) that recruit immune cells, hemocytes, to undead discs. How DUOX is activated in AiP is unknown. DUOX contains two EF-hand motifs which are known to bind Ca^{2+}. Here, we show that intracellular Ca^{2+} levels are increased in the undead AiP model. Downregulation by RNAi of regulators of intracellular Ca^{2+} concentration such as TRP channels (TRPN and TRPM), Storage Operated Calcium Entry (SOCE), and Ca^{2+} effectors like Calmodulin, Calcineurin and CREB-regulated transcriptional coactivator (CRTC) causes modulation in Ca^{2+} concentration and suppresses AiP suggesting that Ca^{2+} plays an important role for AiP. We are currently examining the exact role of the EF-hands for DUOX activation in AiP. In summary, we propose that dynamic modulation of the Ca^{2+} levels activates DUOX potentially via EF-hand binding and thus triggers the production of ROS for the recruitment of hemocytes to induce AiP. Therefore, Calcium serves as the trigger of DUOX activation in the AiP model.

199S **Role of M1BP, a transcriptional pausing factor in JNK-mediated cell death during eye development**
Anuradha V Chimata[1], Hannah Darnell[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,51]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, transcriptional regulation is a fundamental mechanism(s) to regulate differential gene expression. Recently, we have shown that M1BP, a transcriptional pausing factor and a functional homolog of ZKSCAN3, promotes cell survival in developing eyes by suppressing *wingless (wg)*. We performed a forward genetic screen, to identify other targets of M1BP mediated transcriptional repression and may contribute to eye suppression phenotype. We identified members of Jun-amino-terminal-(NH2)-Kinase (JNK) signaling pathway as modifiers of the "no-eye" or "reduced-eye" phenotype of $M1BP^{RNAi}$. We hypothesized that M1BP may promote cell survival in developing eyes by downregulating JNK signaling. Using the GAL4-UAS system, we modulated both JNK signaling components along with downregulation of *M1BP* function and assayed their role in cell survival during eye development. We found that downregulation of *M1BP* results in activation of JNK signaling which in turns activates both apoptosis as well as autophagy. Activation of JNK signaling enhances $M1BP^{RNAi}$ phenotype and downregulation of JNK signaling rescues the $M1BP^{RNAi}$ "no-eye" phenotype. Secondly, blocking cell death or autophagy alone genetically does not completely rescue $M1BP^{RNAi}$ phenotype of "no-eye". Finally, downregulation of *M1BP* along with blocking of both apoptosis and autophagy resulted in a significant rescue of the $M1BP^{RNAi}$ "no-eye" phenotype. Here, we present the role and the mechanism by which transcriptional pausing mechanisms promote cell survival in developing eyes.

200S **JNK and Chk2 Inhibit but E2F1 Promotes Loss of Heterozygosity After Exposure to Ionizing Radiation in Drosophila**
Tin Tin Su[1], Jeremy Brown[21]University of Colorado, [2]University of Colorado Anschutz Medical Campus

Loss of Heterozygosity (LOH) describes a genetic event in which heterozygous mutant cells lose the wild type allele through mutation to become homozygous. LOH can be induced by internal and external mutagens such as ionizing radiation (IR) and can have physiological consequences if the affected gene encodes, for example, a tumor suppressor or an essential function. We have been using *Drosophila melanogaster* as a model to understand mechanisms that modulate LOH incidence after exposure to IR. Our recently described a fluorescence-based LOH reporter based on the QF/QS transcriptional module, which may be used in conjunction with the GAL4/UAS system and applied at multiple developmental stages unlike the traditional adult LOH markers. Using QS inserted at different loci, we demonstrate here that LOH incidence, which is different for different LOH marker used, does not correlate with the distance from the telomere. A focused genetic screen found that Checkpoint Kinase 2 inhibits while E2F1 promotes LOH. Additional data suggest that the role of E2F1 in promoting LOH can be explained by its role in cell proliferation. Combining the QF/QS LOH reporter with QUAS-transgenes to manipulate gene function allowed us to identify mechanisms needed to maintain cells that have already acquired LOH including JNK signaling. These studies reveal previously unknown mechanisms for the elimination of cells with chromosome aberrations.

201S **Extra macrochaetae regulates the Hippo pathway and non-apoptotic caspase control of Notch signaling**

Sudershana Nair, Nick BakerGenetics, Albert Einstein College of Medicine

Extra macrochaetae (Emc) is the sole *Drosophila* representative of the Inhibitor of DNA binding (ID) class of HLH proteins that heterodimerize with proneural bHLH proteins and inhibit them. e*mc* mutations affect many processes not known to depend on proneural genes, as do mammalian ID genes. Previous studies of one example, imaginal disc cell growth, showed that Emc is required to prevent ectopic activity of Daughterless (Da), the other heterodimer partner of proneural proteins, which is expressed in every cell. Da is not generally required in growing imaginal discs, but without Emc, ectopic Da activates the Salvador-Warts-Hippo pathway through *expanded*, reducing DIAP1 activity and growth. To test whether cell survival pathways are involved in other aspects of the *emc* phenotype, we examined the effect of genetically inhibiting caspase activity in *emc* mutant clones in the eye disc. Clones of *emc* mutant cells are known to accelerate the morphogenetic furrow, resulting in premature eye differentiation. Blocking caspase activity completely restored normal morphogenetic furrow progression. Since little cell death was observed, and as ectopic cell death was not sufficient to accelerate the morphogenetic furrow, this must represent a non-apoptotic caspase activity. By testing all the signaling pathways implicated in morphogenetic furrow progression, we established that *emc* clones experience a caspase-dependent increase in Notch signaling, and that this is responsible for accelerating the morphogenetic furrow. This is due to significantly increased expression of Delta, the transmembrane ligand for Notch. Effects of *emc* on other aspects of eye development also depended on caspase activity. Depending on the example, the effects reflected either cell-autonomous inhibition of Notch signaling, due to enhanced cis-inhibition of Notch by Delta, or non-autonomous activation of Notch signaling, due to enhanced trans-activation. Thus, Emc suppresses a Da- and Salvador-Warts-Hippo-dependent non-apoptotic caspase activity that promotes Dl protein expression and is responsible for multiple aspects of the emc mutant eye phenotype. This mechanism might also underlie aspects of the *emc* mutant phenotype in other tissues, some of which are not obviously related to proneural genes.

202S **_Drosophila_ eye model to Study the role of Mnat9 in Alzheimer's Disease related Dementia** Prajakta Deshpande[1], Emily Snider[1], Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,51]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

Alzheimer's Disease (AD), a progressive neurodegenerative disorder, is manifested as extracellular accumulation of amyloid-beta-42 (Aβ42) plaques and intracellular accumulation of neurofibrillary tangles (NFTs) due to hyperphosphorylation of tau that results in destabilization of microtubules. Targeted misexpression of human Aβ42 (GMR>Aβ42) in retinal neurons of developing *Drosophila* eye results in Aβ42 plaque(s) formation, extensive neurodegeneration and mimics AD like neuropathology. However, the underlying mechanism(s) for Aβ42-mediated neurodegeneration have not been fully understood. In a forward genetic screen, we identified *N-acetyltransferase 9* (*Mnat9*) as one of the genetic modifiers of GMR>Aβ42 neurodegenerative phenotype. Mnat9 is known to stabilize microtubules by inhibiting c-Jun-N- terminal kinase (JNK) signaling. The neurodegenerative phenotype of GMR>Aβ42 is rescued by gain-of-function of *Mnat9* whereas loss-of-function of *Mnat9* exhibits converse phenotype of enhanced neurodegeneration. Human Mnat9 also suppresses Aβ42-mediated neurodegeneration suggesting the functional conservation. Surprisingly, Mnat9 neuroprotective function is independent of its acetylation activity. We found that Mnat9 downregulates JNK signaling pathway, which is involved in rescuing neurodegenerative phenotypes seen in GMR>Aβ42 background. Here, we propose a new neuroprotective function of Mnat9 in downregulating JNK signaling pathway to ameliorate Aβ42-mediated neurodegeneration.

203S **Effects of proteotoxic stress in D. melanogaster oocytes and embryos on viability, fertility and transcription.** Natalia TamarinaEcology and Evolution, University of Chicago

Environmental stresses such as heat, cold and hypoxia can affect embryonic development and gene expression in *Drosophila*, but the effects of proteotoxic stress are not well studied in the embryo. Stress of the cell proteome occurs with conditions that interfere with proper protein folding, such as misfolding mutations, mutations affecting molecular chaperones, perturbation of endoplasmic reticulum calcium stores, and others. Previously we created a Drosophila model system for studies of cell stress by ectopically expressing human insulin gene hINS and its misfolding mutation variant hINS(C96Y) (Park et al, 2014). When expressed in fly imaginal discs, the mutated gene generates Unfolded Protein Response (UPR) and proteotoxic stress conditions, which affects the balance of cell proliferation and death, similar to its effects in mammalian systems.

In current study I explored embryonic and ovarian expression of hINS and hINS(C96Y), to probe for effects of proteotoxic stress on viability (survival to adulthood) and on female fertility. Maternally expressed *nanos*-GAL4 was used to drive the expression of both insulin genes in the *D. melanogaster* embryo by crossing GAL4-expressing females with males heterozygous for UAS-gene. The relative viability of flies with different genotypes was studied in F1. The expression of mutated insulin gene UAS-hINS(C96Y) at embryonic stage generated 23.3% fewer adult males (n=400 $p<0.01$), while females expressing this gene did not show significant loss of viability (n=400 $p=0.22$).

We also studied the effects of *nanos*-GAL4 driven hINS and hINS(C96Y) expression in oocytes. Co-expression of *nanos*-GAL4 in ovaries with UAS-hINS or UAS-hINS(C96Y) led to corresponding 18.3% and 9.3% reduction of female fertility compared to control (n=12,000, $p<0.01$). The results also show that female offspring is relatively less sensitive to the presence of ectopically expressed proteins compared to males, especially in case of hINS(C96Y) expression, although this difference did not reach statistical significance.

I hypothesize that maternal effects of proteotoxic stress can be mediated through damaging effects on transcription in early embryogenesis. To approach this question, the time- and spatially-resolved transcription of embryonically active *hunchback*-P2P-LacZ reporter gene is being tested in hINS/hINS(C96Y) -stressed embryos using live method of monitoring with MS2/PP7 fluorescent tagging and live confocal imaging.

204V **Die or eat your neighbors: Induction of apoptosis or phagocytic activity in epithelial cells is a consequence of who dies first** Keren Yacobi-Sharon, Eli AramaDepartment of Molecular Genetics, Weizmann Institute

Apoptosis is executed by the action of caspase proteases, which when activated above a lethal threshold, lead to a cell death and rapid clearance by professional (macrophages) or non-professional (neighboring cells) phagocytes. Phagocytosis of apoptotic cells involves recognition of the dying cell, internalization into phagosomes, and final degradation following fusion of the phagosomes with lysosomes. While macrophages are cells specialized in clearing apoptotic cells and other large particles, some nurse cells are also capable of acquiring a phagocytic activity, such as glia and Sertoli cells that clear dying neurons and germ cells, respectively. However, much less is known about how some cells within a tissue composed of similar cells are selected to acquire phagocytic properties. Here, I present our unpublished data about the mechanisms involved in the acquisition of phagocytic activity in a simple tissue of largely homogenous epithelial cells experiencing an identical stress. We show that following ionizing irradiation, some epithelial cells within the *Drosophila* wing imaginal discs do not die, but become positive to Lysotracker, a processes that requires the main phagocytic receptor Draper (CED-1 homolog). Indeed, while the cells still die in the absence of Draper, apoptotic clearance is severely impaired. Significantly, we also show that acquiring phagocytic activity requires a non-autonomous signal emanating from the dying adjacent cells. Overall, our data supports a model in which cells that stochastically first reached the lethal threshold of caspase activity, dictate survival and a phagocytic fate in neighboring cells that lag behind in reaching that threshold.

205V **Utilizing Live Cell Imaging in Drosophila melanogaster Salivary Glands to Determine if Resveratrol Treatment Activates Heat Shock Factor DNA Binding** Martin Buckley, Nichole Webb, Tyra Skalos, Stacy HrizoSlippery 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.

206V **Mitochondrial membrane associated Spoonbill protein modulates JNK mediated apoptosis in *Drosophila*** Rituparna Das, Ashim Mukherjee, Mousumi MutsuddiMolecular and Human Genetics, Banaras Hindu University

Protein Kinase A Anchoring Protein (AKAPs) are a family of scaffolding proteins which provides a physical conduit that regulates signaling cascades. These proteins regulate the spatial and temporal dynamics of the signaling pathways by interacting with the integral components of various pathways. *Drosophila* Spoonbill is one such putative PKAAP protein. This gene was initially identified by us as a suppressor of non-coding trinucleotide repeat expansion associated Spinocerebellar Ataxia 8 neurodegeneration. Here, we report that Spoonbill positively regulates Eiger induced apoptosis in *Drosophila*. Binding of Eiger, the *Drosophila* TNFα ligand with its receptor Wengen results in activation of JNK signaling. Assessment of JNK pathway in Eiger induced condition show that modulating the Spoonbill levels alters the activation of JNK and its downstream targets. Epistatic analysis using various components of JNK pathway, show that Spoonbill acts downstream to the kinases of JNK pathway. Additionally, Spoonbill could ectopically activate the JNK in a context specific manner, indicating that Spoonbill can directly regulate the JNK signalling. For exploring the underlying mechanism of this regulation, the interaction was investigated between Spoonbill and Basket, the *Drosophila* JNK. Interestingly, Spoonbill was found to localize in the same subcellular compartment as Basket. Co-immunoprecipitation assay revealed that Spoonbill is part of the same multiprotein complex as Basket. Further, Spoonbill was found to be vital for Parkinson's Disease associated JNK activation which affected the motor ability. Thereby, we conclude that Spoonbill protein facilitates the localization of JNK at a particular cellular niche that is critical for its activation. Based on our observation and previous literature it is known that Spoonbill is localized on outer mitochondrial membrane (OMM). Perhaps, the localization of *Drosophila* JNK defined by Spoonbill protein on or in the vicinity of the OMM is critical for its activation. Possibly, Spoonbill work with other scaffolding proteins to provide a physical means for signal transduction of JNK pathway. Further investigation into this interaction will, in the future, aid in a better understanding of the crosstalk between various signaling pathways and in establishing a platform for the therapeutic development of neurological diseases like the Parkinson's.

207T **Investigating the molecular basis for host-microbe specificity in the *Drosophila melanogaster* gut** Kevin Aumiller[1,2], Karina Gutierrez-Garcia[2], Sneha Agrawal[1], Ann Deng[1,2], Xincheng Yuan[1], Yizhan Guo[1,2], William Ludington[1,21]Johns Hopkins University, [2]Carnegie Institution for Science

Animals have evolved to form symbiotic relationships with resident gut microbiota that are both stable and host-specific. Characterizing the genetic determinants of host specificity is important for the study of gastrointestinal diseases such as diabetes and obesity, which have been shown to be influenced by the microbiota. *Lactiplantibacillus plantarum* (*Lp*) is a highly prevalent gut symbiont that exhibits probiotic properties and a diverse host range that includes mammals and the fruit fly *D. melanogaster*. We isolated a strain of *Lp* (WF) from *D. melanogaster* that establishes a highly stable and spatially defined niche within the foregut. Using this system, we developed a model to identify the determinants of host-specific colonization through *in vitro* evolution of WF. Through long-read sequencing, we identified a genomic island consisting of 27 genes grouped into eight gene blocks that are deleted sequentially via transposase activity during evolution. Complete loss of the island was observed after 51 days (~500 generations) in several replicates, with these mutants exhibiting (I) a sharp decrease in colonization efficiency, (II) a reduced spatial specificity for the foregut niche and (III) an acute selective disadvantage when placed in competition with wild-type in the fly gut. Phenotypic characterization of several evolved mutants revealed that the island encodes a pair of large serine rich repeat adhesins (SRRPs) that drive selection for strong colonizers, but the genes are rapidly lost without selection for colonization during *in vitro* growth. Other *Lp* strain isolates from *D. melanogaster* that encode allelic variants of the SRRPs showed different colonization efficiencies and spatial localization in the gut, suggesting that these adhesins function to establish distinct sub-niches within the foregut. Biochemical analysis of recombinant SRRP binding domains revealed that the adhesins bind several glycoproteins specifically expressed in the foregut, with current evidence suggesting that differential affinity for glycoconjugates underlies sub-niche construction. From our findings, we propose that the host environment selects for specificity of colonization by modulating bacterial adhesion through a mechanism that supports natural variance in colonization phenotypes. We currently aim to identify the host-expressed binding partners of the SRRPs to further investigate the mechanisms by which the host establishes and maintains specific host-microbe associations.

208T **Investigating the role of microRNAs (miRNAs) in *Drosophila* aging antiviral immunity** Max Y Lu, Amber

Thibeaux, Lakbira A Sheffield, Stanislava ChtarbanovaDepartment of Biological Sciences, The University of Alabama

microRNAs (miRNAs) are a class of non-coding RNAs which act as post-transcriptional regulators of gene expression. Possessing a plethora of targets, miRNAs play roles in many biological processes including development, aging and innate immunity. A gap in knowledge exists in our understanding of how miRNAs orchestrate innate antiviral responses in aging hosts. As older individuals are more susceptible to viral infections, uncovering mechanisms of this regulation could identify novel potential targets to improve infectious outcomes among the elderly. Using a *Drosophila melanogaster*-Flock House virus (FHV) host-virus model, we have previously shown that aging in flies is associated with a robust transcriptional response and results in decreased survival of FHV infection due to impaired tolerance. Here, we used small RNA-seq analysis to identify differential changes in miRNA expression in young and aged cohorts 48h following FHV infection. We found that younger flies differentially expressed more miRNAs than older flies. Subsequent Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analysis on targets of differentially expressed miRNAs showed neurogenesis enrichment for both young and aged infected flies and reproduction and metabolic process enrichment for aged infected flies. Using the GAL4/UAS system we ubiquitously drove expression of miRNA sponge constructs to knockdown (KD) 17 candidate miRNAs and examined survival outcomes after FHV infection as a function of age and sex. We found that aged flies generally displayed increased susceptibility compared to younger counterparts and that in every line examined females also outlived age-matched males. miRNA KD also had a greater age-dependent effect on survival in males compared to females. In comparison to controls, KD of miR-311 led to significantly decreased survival of FHV of aged males, associated with comparable virus titers across samples. Taken altogether, our work provides insights into the role of miRNAs during FHV infection and identifies miRNAs with potential roles in innate immunity in the context of aging. In particular, it highlights miR-311 as one miRNA responsible for regulating FHV tolerance.

209T **Brain innate immune activation following bacterial infection in *Drosophila melanogaster*** Sameekshya Mainali[1], Paige Magid[2], Lauren Harper[1], Jordan Grammer[1], Kaitlin Berry[1], Elizabeth Kitchens[1], Stanislava Chtarbanova[11]University of Alabama, [2]Bates College

Excessive inflammatory responses resulting from infections of the central nervous system (CNS) could have deleterious consequences on brain health and result in neurodegenerative disease. However, the exact cellular and molecular mechanisms leading to disease manifestation and progression in such contexts are unclear. *Drosophila melanogaster* represents an ideal genetically tractable system to investigate the processes associated with infection-mediated neurodegeneration because both the mechanisms of neural development and function and innate immunity are highly conserved from flies to humans. Previous work in flies has shown that bacterial brain injection results in progressive, age-dependent neurodegeneration and impaired locomotor activity. We hypothesize that sub-lethal brain infection with Gram-positive (*Micrococcus luteus*) and Gram-negative (*Escherichia coli*) bacteria activates the Toll and Immune Deficiency (IMD) innate immune pathways, respectively, and that specific cell types within the brain mount these responses. In comparison to controls poked with a sterile needle, direct brain infection with *E.coli* and *M.luteus* of *Drosophila* lines carrying innate immunity GFP reporters led to GFP expression in different subtypes of glial cells 24h post-treatment. We assayed *Drosomycin* (*Drs*) or *Diptericin B* (*DiptB*) expression via quantitative reverse transcription PCR in mutants for components of the Toll (*MyD88*) and IMD (*Dredd*) pathways, respectively using RNA isolated from dissected brains. Like a systemic infection, in *wild-type* controls, we found a significant difference in brain-specific *Drs* expression between the sterile and bacterially injured flies, which was suppressed in *MyD88* mutants. This suggests that *Drs* is likely regulated by canonical upstream components of the Toll pathway in the brain. Similarly, preliminary results show that in comparison to controls, *DiptB* expression in the brain is suppressed in *Dredd* mutants infected with *E. coli*. Currently, we are comparing *Drs* and *DiptB* expression in the brain following thorax pricking, as well as comparing gene expression kinetics between direct brain infection and systemic infection. Also, we are evaluating whether glial cells acutely activate NF-kB innate immune responses, which may or may not persist over time. A better characterization of these responses will advance our knowledge of how non-lethal brain bacterial infection results in innate immune activation and in neurodegeneration over time.

210T **Expression of retrotransposons contributes to aging in *Drosophila*** Blair K Schneider[1], Shixiang Sun[1], Moonsook Lee[1], Wenge Li[2], Nicholas Skvir[3], Nicola Neretti[3], Jan Vijg[1], Julie Secombe[11]Genetics, Albert Einstein College of Medicine, [2]cell biology, Albert Einstein College of Medicine, [3]Molecular biology, Cell biology and Biochemistry, Brown University

Retrotransposons are a class of transposable elements capable of self-replication and insertion into new genomic

locations. Across species, the mobilization of retrotransposons in somatic cells has been suggested to contribute to the cell and tissue functional decline that occurs during aging. Retrotransposon expression generally increases with age, and *de novo* insertions have been observed to occur during tumorigenesis. However, the extent to which new retrotransposon insertions occur during normal aging and their effect on cellular and animal function remains understudied. Here we use a single nucleus whole genome sequencing approach in *Drosophila* to directly test whether transposon insertions increase with age in somatic cells. Analyses of nuclei from thoraces and indirect flight muscles using a newly developed pipeline, Retrofind, revealed no significant increase in the number of transposon insertions with age. Despite this, reducing the expression of two different retrotransposons, *412* and *Roo*, extends lifespan, without increasing stress resistance. This suggests a key role for transposon expression and not insertion in regulating longevity. Transcriptomic analyses revealed similar changes to gene expression in *412* and *Roo* knockdown flies and highlighted potential changes to genes involved in proteolysis and immune function as potential contributors to the observed changes in longevity. Combined, our data show a clear link between retrotransposon expression and aging.

211T **Uncertainty favors an induced immune response to infection** Danial Asgari[1], Richard P Meisel[2], Alexander J Stewart[31]University of Houston, [2]Biology, University of Houston, [3]University of St Andrews

Organisms can use constitutive or induced defenses against natural enemies, such as pathogens, parasites, and herbivores. Constitutive defenses are constantly on, whereas induced defenses are only activated upon exposure to an enemy. Constitutive and induced defensive strategies each have costs and benefits, which can affect the type of defense that evolves for a particular threat. Previous modeling that compared induced and constitutive defenses relied on conceptual models that lacked mechanistic details about host defense, did not consider pathogen proliferation rates, or lacked both features. To address this gap, we developed a detailed mechanistic model of the well-characterized Drosophila melanogaster immune response to bacteria with different proliferation rates and environmental distributions. Our model includes a system of differential equations that capture the D. melanogaster immune signaling network. We used our model to evaluate the factors favoring the evolution of constitutive and induced defenses by comparing the fitness of each strategy upon stochastic encounters of flies with bacteria. We found that induction is generally preferred in environments where fly-bacteria interactions are less frequent. We further show that the relative fitness of the induced defense depends on the interaction between the bacterial proliferation rate, density, and the spatial distribution of the bacteria. Also, our model predicts that the specific negative regulators that optimize the induced response depend on the bacterial proliferation rate. Finally, we show that uncertainty over bacterial encounters favors the evolution of an induced immune response. Uncertainty in our model can arise from heterogeneous distributions of bacteria, as well as fluctuations in the density or patchiness of the bacterial population. This result provides evidence that environmental uncertainty favors induced defenses.

212T **Characterization of cross-species transmission of *Drosophila melanogaster* Nora virus** Ella Buhlke[1], Alexis Hobbs[1], Sunanda Rajput[1], Blase Rokusek[1], Darby J Carlson[1], Chelle Gillan[2], Kimberly A Carlson[11]Biology, University of Nebraska at Kearney, [2]Central City Senior High School

Drosophila melanogaster Nora virus (DmNV) is a novel picorna-like virus first characterized in 2006. Since then, Nora virus has been detected in several non-*Drosophila* species, including insects in the Orders Hymenoptera, Lepidoptera, Coleoptera, and Orthoptera. The objective of this study was to determine if DmNV could infect individuals of other species of invertebrates besides *D. melanogaster*. The presence of DmNV in native invertebrates and commercially available stocks was determined. Laboratory-reared *D. yakuba, D. mercatorum*, *Gryllodes sigillatus*, *Tenebrio molitor*, *Galleria mellonella*, and *Musca domestica* were intentionally infected with DmNV. In addition, native invertebrates were collected and *D. melanogaster* stocks were purchased and screened for DmNV presence using reverse transcription-polymerase chain reaction (RT-PCR) before being intentionally infected for study. All *Drosophila* species and other invertebrates, except *M. domestica*, that were intentionally infected with DmNV ended up scoring positive for the virus via RT-PCR. DmNV infection was also detected in three native invertebrates (*Spilosoma virginica*, *Diplopoda*, and *Odontotaenius disjunctus*) and all commercially available stocks tested. These findings suggest that DmNV readily infects individuals of other species of invertebrates, while also appearing to be an endemic virus in both wild and laboratory *D. melanogaster* populations. The detection of DmNV in commercially available stocks presents a cautionary message for scientists using these stocks in studies of virology and immunology. The project described was supported by grants from the National Institute for General Medical Science (GM103427 & 1U54GM115458).

213T **Potential for viral neuro-invasion in Nora virus Infected *Drosophila melanogaster*** Blase Rokusek, Shalie Sklenar, Luke Hamilton, Britney de Leon, Sunanda Rajput, Darby J. Carlson, Nicholas Hobbs, Kimberly A. CarlsonBiology,

University of Nebraska at Kearney

Drosophila melanogaster Nora virus (DmNV), a positive-sense single stranded RNA virus in the family Picornaviridae, was first described less than two decades ago when it was found to infect *D. melanogaster*. At the time of its discovery, DmNV replication appeared to be largely confined to the gut, with limited evidence that productive infection occurred elsewhere. However, since that time, there is evidence to suggest that DmNV could infect other regions of the fly anatomy outside of the gut. The purpose of the present study was to determine whether DmNV is capable of invading the heads and brain of *D. melanogaster*. To begin the investigation, fly heads were removed from chronically infected stocks (as well as from uninfected controls) and RNA and protein extracted. Reverse transcriptase-polymerase chain reaction (RT-PCR), utilizing gene specific primers for the DmNV *open reading frame 1* (*ORF1*), was used to analyze the RNA extracts. SDS/PAGE and Western blot analyses were performed on the protein lysates using anti-VP4b (DmNV capsid protein) antibody. The results of these experiments showed that both DmNV genomic material and DmNV capsid protein can be isolated from the heads of the infected flies, respectively. Thus, we can conclude that DmNV reaches the heads of fruit flies during the course of infection. However, the question remains as to whether the virus infects the brain tissue itself. To answer this question, RNA Fluorescence *in situ* Hybridization (FISH) experiments on whole brains dissected out of DmNV-infected and uninfected flies are currently underway in our lab. We have designed the RNA FISH probes to be complimentary to sequences of the DmNV *ORF4*. It is still too early to draw any definitive conclusions from the preliminary results of these experiments. Confirmation of virus within brains of DmNV-infected flies would further characterize this endemic *D. melanogaster* virus that chronically infects laboratory stocks. Further, should we be able to show that the RNA FISH method works for detection of viral genomes within *D. melanogaster*, this method could prove valuable for future work involving viral infection in fruit fly models. The project described was supported by grants from the National Institute for General Medical Science (GM103427 & 1U54GM115458).

214T **Selfish signaling: metabolic reprogramming during immune response** Ellen McMullen, Lukas Strych, Tomas DolezalUniversity of South Bohemia

JAK/STAT signaling is a key regulator of immune response; conserved from flies to humans. Drosophila larvae rely on JAK/STAT signaling to fight parasitoid wasp infection (*Leptipilina boulardi*) through the secretion of Upd2 and Upd3 cytokines. Upon the recognition of a wasp egg, hemocytes release Upd2 and Upd3, activating JAK/STAT signaling in muscles and allowing the differentiation of hemocytes into lamellocytes (activated immune cells). This is mediated by the redirection of nutrients away from muscles in favor of the immune system, supplying immune cells with sufficient energy to mount an effective immune response. Deletion of Upd2 and Upd3 leads to significant reduction in lamellocyte number and reduced survival.

The systemic nutritional shift that occurs as a result of parasitoid wasp infection causes carbohydrates to be directed away from non-immune tissues in favour of 'selfish' immune cells. This metabolic shift appears to be mediated by suppression of insulin signaling (IS) in non-immune organs, such as muscles. Our findings show lower levels of pAkt in muscles during infection and an increase in the relative expression of 4E-PB, indicating IS suppression. However, these changes are not observed in Upd mutants upon infection. Additionally, the inhibition of Insulin resistance in the muscle tissue of Upd mutants leads to a rescue in lamellocyte number.

215T **Senescent cells promote tumorigenesis through non-autonomous interactions with hemocytes in a larval model of colorectal cancer.** Ishwaree Datta, Erdem BangiBiological Science, Florida State University

Cellular senescence is a highly conserved and dynamic process that occurs in response to various biological stresses such as aging, DNA damage, oxidative stress, chemotherapy, tissue repair and remodeling. Senescent cells display irreversible cell cycle arrest, mediated by the Cyclin Dependent Kinase (CDK) inhibitor, p21. Although they do not undergo cell division, these cells remain metabolically active and secrete certain critical molecules to dynamically interact with their microenvironment. The presence of senescent cells is especially critical in the context of cancer and is termed oncogene-induced senescence. Although in certain cases, senescence acts as a defense mechanism against tumorigenesis, the prolonged presence of senescent cells within tumors has been observed to aid in cancer progression as well as relapse of more invasive malignancy following chemotherapy. While senescent cells have been well characterized in cultured cells, their small number in tissues makes mechanistic in vivo studies of senescence challenging. As a result, how various triggers of senescence are integrated in vivo to induce senescence is unclear.

We have recently established a 4-hit colorectal cancer (CRC) model in the developing larval hindgut epithelium by targeting APC, KRAS, P53 and PTEN, four genes frequently altered in human colon tumors. In this transformed

background, we identified a small number of cells exhibiting multiple senescence features, including the upregulation of the Drosophila p21 Dacapo (Dap). Elimination of these Dap-positive cells genetically led to the significant rescue of the tumor phenotype, indicating a tumor-promoting role for senescent cells. Further, oral administration of the senolytic drugs Navitoclax and Fisentin, which are known to kill senescent cells, also suppressed transformation. We found that the senescence program in the transformed tissue is triggered by a complex mechanism that incorporates input from AKT, JNK and DNA damage response pathways. We also determined that tumor-promoting effects of senescence are mediated by non-autonomous interactions between senescent cells, their non-senescent transformed neighbors and the immune system. These findings suggest a novel mechanism for the tumor-promoting role of senescent cells and could lead to a promising and novel avenue for treating CRC patients.

216T **Mechanisms of immune regulation by Ecdysone and Juvenile Hormone** Scott A Keith[1], Vanika Gupta[1], Dana Vargas Solivan[2], Brian Lazzaro[21]Entomology, Cornell University, [2]Cornell University

Circulating hormones simultaneously impact varied physiological functions through effects on gene expression and cell biology. In Drosophila, 20-hydroxyecdysone (20E) and juvenile hormone (JH), regulate development, metabolism, and reproduction. These hormones and their cognate receptors have reciprocal effects on immunity, with 20E potentiating and JH suppressing innate immune responses. Yet little is known about the molecular bases of these effects or the scope of additional microbe-responsive physiologies controlled by these hormones. We are investigating both of these endocrine systems with the ultimate goal of understanding how they might interact to modulate host physiology and immune responses during infection. JH mediates an immune-reproduction tradeoff wherein mated females exhibit decreased infection resistance in a JH-dependent manner, however we know almost nothing about the underlying mechanisms. 20E has been shown to activate immunity in developmental and infection contexts, but the exact regulatory mechanisms of this activation remain unclear. We found that mating increases infection susceptibility in part by leading to reduced translational capacity of the fat body, the primary immune tissue. We are currently testing the hypothesis that JH signaling is responsible for this strain on cellular resources in the fat body. In parallel, we show that ecdysone receptor (EcR) function in the fat body is required for survival of bacterial infection, consistent with prior reports. We hypothesize that 20E-EcR signaling controls a hierarchical gene regulatory network (GRN) in the fat body that sustains immune activation. To test this hypothesis and begin to construct a GRN, we are using RNA-seq to identify 20E-dependent, infection-induced gene expression changes in the fat body, and CUT&RUN analysis to determine which differentially expressed genes represent direct versus indirect EcR targets. Through this work, we ultimately aim to better understand hormone-mediated regulation of the physiological balance of development, metabolism, reproduction, and immunity in the context of host-microbe interactions.

217T **Plasma membrane rupture protein Ninjurin A controls susceptibility of *Drosophila melanogaster* to Invertebrate Iridescent Virus 6 (IIV6) infection in a *Turandot*-independent manner.** Molly Murphy, Neal SilvermanMedicine, University of Massachusetts Chan Medical School

Invertebrate Iridescent Virus 6 (IIV6) has been shown to suppress the Toll and Imd pathways as well as the antiviral RNAi pathway in *Drosophila melanogaster*. Despite this widespread immunosuppression, *Drosophila* mount a response to IIV6 infection through the induction of unpaired (UPD) cytokines which in turn drive *Turandot (Tot)* gene expression. The *Tot* family of genes have been implicated in the *Drosophila* response to a wide variety of cellular and environmental stresses, indicating this UPD-triggered cascade may have a more general stimulus, rather than recognizing a specific viral product. While working towards illuminating this pathway further, we discovered that *Ninjurin A (NijA),* encoding a protein recently linked to plasma membrane rupture in both *Drosophila* and mammals, is important in host defense against IIV6. While $NijA^{D3}$ null mutants did not alter the induction of *Tot* genes, these animals were significantly more susceptible to IIV6 infection. These findings present interesting questions regarding the role of *NijA* and plasma membrane rupture in anti-viral host defense, as well as on the molecular mechanisms downstream of viral infection to induce *Tot* genes.

218F **Regulation of the Imd Pathway by Steroid Hormones and Immune Crosstalk** Bao Ho[1], Peter Nagy[2], Varada Abhyanka[3], Nicolas Buchon[2], Neal Silverman[11]University of Massachusetts Chan Medical School, [2]Cornell University, [3]Princeton University

The Imd innate immunity pathway in *Drosophila melanogaster* is a powerful model for the study of innate immunity. While the core of this bacterial-sensing pathway is a well-established NF- κB (*Relish*) activating response, how it is connected to or regulated by other pathways, especially steroid hormone signaling is a key knowledge gap in understanding the physiological control of innate immunity. Previously, we demonstrated that *PGRP-LC*, which encodes

the upstream peptidoglycan sensor of the Imd pathway, is an indirect target of 20-Hydroxyecdysone regulation. Interestingly, a recent paradigm shift in steroid hormone biology argues that the trafficking of Ecdysone across cell membranes requires a specific importer, known as *EcI*. New genome-wide analysis of immune responsive gene expression suggests that expression of *EcI* may in fact be immune regulated. In this study, we continue to probe the role of Ecdysone and its transport in the regulation of the innate immune response.

In the same transcriptome analysis, *Diedel*, a known negative regulator of the Imd pathway, is up-regulated by several immune stimuli. *Diedel* is activated to varying degrees following Imd, Toll or Jak/Stat activation. This suggests an Imd regulatory module, through *Diedel*, common to multiple immune pathways. Interestingly, induction of *Diedel* expression requires *Relish*, with all stimuli, and in some cases also requires the JAK/STAT activating cytokines UPD2/3. In this study we continue to investigate the regulation and function of *Diedel* in the immune response.

219F **The role of environmental fermentation and host metabolic genotype on the Drosophila gut microbiome** Nitin Bansal[1], Kailee Ward[2], Kristi Montooth[3], Clay Cressler[3], Ian Keesey[31]Biological Sciences, University of Nebraska-Lincoln, [2]University of Nebraska-Lincoln, [3]School of Biological Sciences, University of Nebraska-Lincoln

Organisms rely on gut microbiota for proper health. Thus, it is important to understand the factors that lead to changes or stability in gut microbiota. Diet affects microbiome composition in Drosophila (Obadia et al. 2018) and dietary microbes can also affect development and lifespan in flies (Keebaugh et al. 2018). The goal of my research is to understand how interactions between nutrient environment and host metabolism impact gut microbiomes and the effect on life history among different species and genotypes of Drosophila. To address this question, we have exposed flies from two species groups of Drosophila that vary in ecology – *D. suzukii, D. subpulchrella, D. biarmipes,* and *D. takahashii* from the Suzukii group, and *D. sechellia, D. simulans, D. melanogaster,* and *D. erecta* from the Melanogaster group – to a gradient of natural fermentation resulting from blueberries in their diet. To test whether host metabolic variation can impact microbiome stability across levels of dietary fermentation, we exposed a set of well characterized mitochondrial-nuclear genetic variants to the same diets. DNA from hosts and their diets was extracted for bacterial 16s rRNA gene sequencing to estimate the relative abundance of bacterial groups in these samples. In addition, the DNA virus and phage component of the gut microbiome will be characterized using metagenomic sequencing from Oxford Nanopore MinIONs. These data will inform our understanding of the relationship between host genotype, metabolism, and fermentation environment on microbiome stability and its impact on host life history traits and ecological divergence in Drosophila. This research is part of the newly established NE insect MicroBiome initiative (NiMBi) collaborative research group investigating how host-environment-microbiome interactions contribute to host plant shifts in Drospohila, the Colorado potato beetle, and the soybean gall midge.

220F **Single Cell transcriptomic analysis of hemocytes in *Drosophila* overgrowth/tumor models** Prathibha Yarikipati, Andreas BergmannMolecular Cell and Cancer Biology, University of Massachusetts Medical School

Many genetic models of hyperplastic and neoplastic tumorous growth in *Drosophila* imaginal discs are characterized by the recruitment and activation of hemocytes, macrophage-like cells of the immune system that may have tumor-promoting or tumor-suppressing functions. Both conditions also contain an increased number of hemocytes in circulation compared to the respective controls and have intact lymph glands unlike what is observed in microbial infections. The origins and transcriptional profiles that drive these hemocytes to the site of overgrowth/tumors are not well known. Here, we used single-cell RNA sequencing (scRNA-seq) on 10X-genomics platform to characterize circulating hemocytes across genetic models of hyperplastic growth (apoptosis-induced proliferation (*ey>hid*; *p35*)) and neoplastic tumors ($RasV^{12}$; $scrib^{2}$) in larval eye imaginal discs. We resolved ~84,000 live sessile and circulating hemocytes from larvae bearing hyperplastic and neoplastic tumors and their respective controls into 22 different cell clusters. We did not find any significant differences in abundance or transcriptional profile of crystal cells, lamellocytes and antimicrobial peptide clusters compared to previously published scRNA-seq data of hemocytes. In contrast, several of the plasmatocyte clusters are strongly enriched in larvae bearing hyperplastic and/or neoplastic tumors, while other clusters are strongly reduced in these backgrounds. Further, we have identified regulatory factors for each of these clusters and analyzed their origins through pseudo-temporal ordering. Our scRNA-seq analysis reveals an unexpected degree of plasmatocyte plasticity in an overgrowth/tumorous environment and provides a specific transcriptional profile for understanding systemic immune responses in overgrowth and tumor models of *Drosophila*. These data will be presented at the conference.

221F **Sex-differential immune gene expression and immune response in *Drosophila melanogaster and***

***Drosophila simulans*.** MD Mursalin Khan, Madeline Gwin, Carter Zittrouer, Rita M. GrazeBiological Sciences, Auburn University

Sex dimorphism in the immune response is thought to arise from fundamental life history differences between females and males. Infection with different pathogens results in different modes of immune sex dimorphism, likely due to the specific disease-causing mechanisms or host-pathogen interactions. It is unclear how these differences evolve and if sex differences in the immune response that have been observed in *D. melanogaster* are conserved in related species. Even if the mode of sex differences is conserved, species may differ quantitatively in the relative expression of immune response genes in males and females. In this study, we aim to understand whether two closely related Drosophila species show conserved sex dimorphism in the immune response to gram-negative or gram-positive bacterial infection. We investigate the relationship between sex dimorphism in the regulatory response and different measures of infection outcome in *Drosophila melanogaster* and *D. simulans* with gram-negative, *Providencia rettgeri* (PRET) and gram-positive, *Enterococcus faecalis* (EFAS) infection in each sex. Differential gene expression was assayed by RNASeq, survival rate, bacterial load, and bacterial load upon death (BLUD) were measured. Survival upon infection with gram-negative (PRET) showed greater differences between the sexes in *D. melanogaster* relative to *D. simulans*. For gram-positive (EFAS) bacterial infection, both species had similar differences between the sexes, and these differences were smaller than those observed for PRET in *D. melanogaster*. Differential gene expression analysis elucidates the corresponding sex dimorphism upon infection with gram-negative (PRET) and gram-positive (EFAS) in each species. Overall, our results indicate some sex-differences in the immune response for both types of infection, but that sex dimorphism in the immune response has diverged between these two closely related Drosophila species.

222F **Gustatory receptors regulate metabolism and immunity** Jin Seo[1], Eunji Yoon[2], Tucker Hopkins[21]Biology, Rogers State University, [2]Rogers State University

Metabolism and immunity, the two most conserved and fundamental processes, are coordinated. Maintaining high immunity is beneficial to a host during microbial infection but requires a tremendous amount of stored energy, thereby significantly losing nutrient storage. Conversely, decreased energy storage and small body size are often linked to enhanced immunity. Although metabolism and immunity are closely coordinated, the signaling molecules connecting metabolism and immunity are not well understood.

Insect gustatory receptors (GRs) detect nonvolatile compounds and regulate behavioral preferences on food selection, mate choice, and site selection for egg deposition. In the *Drosophila melanogaster* genome, the GR family contains 68 members. Expression of the GR family is largely restricted to the neurons in the labellum, antenna, and legs to detect taste-provoking molecules such as sucrose and alkaloids. However, some GRs have been demonstrated to play roles in the tissues beyond tastants-sensing neurons. For instance, mouse taste receptors are expressed in digestive tracts and regulate appetite and insulin secretion. Knocking out of sweet receptors significantly decreased body weight and fat content. Similarly, we have previously demonstrated that multiple GRs have regulated body fat in the fat body-specific manner. Since the fat body is equivalent to adipose tissue and liver in the vertebrates, we hypothesized that the GR family might regulate immunity as well.

Melanotic masses often result from activation of the immune pathway. Interestingly, knockdown of *Gr47b* resulted in melanotic masses in their abdomen; further, it upregulated expression of antimicrobial peptides (AMPs), which inhibit a wide range of bacteria, fungi, parasites, and viruses. In mammals, immune system overactivity often induces adipose tissue inflammation and metabolic abnormalities. Agreeing with the notion, the Gr47b mutant flies significantly altered levels of circulating carbohydrates in hemolymph.

223F **Regulation and function of SLC46 Family Member CG15553 in Immunity** Benedetta D'Elia[1], Varada Abhyankar[2], Bao Ho[1], Jafira Johnson[3], Neal Silverman[31]Medicine, UMass Chan Medical School, [2]Princeton University, [3]UMass Chan Medical School

Drosophila melanogaster has long been successfully employed as a model for the study of innate immunity - namely, the first line of defense against microbial infection, highly conserved across species. A thorough understanding of innate immune signaling pathways is critical to elucidate the mechanisms underlying immune diseases, to translate them into mammalian systems, and develop new therapies to treat them.

In *Drosophila*, bacterial infections are sensed by two NF-κB pathways, which orchestrate immune recognition and signal transduction. Activation of the Toll and the immune deficiency (Imd) pathways triggers the production of antimicrobial

peptides (AMPs) to counter bacterial infections. While both pathways are triggered by peptidoglycan (PGN), Imd is potently activated by DAP-type PGN, which is common to Gram-negatives and certain Gram-positive bacilli and is recognized by the peptidoglycan recognition proteins PGRP-LC in the extracellular space and PGRP-LE in the cytosol. The first critical step in the cytosolic recognition of bacterial peptidoglycan is transport of small PGN fragments (muropeptides) into the cell. How DAP-muropeptides reach the cytosol is a major gap in knowledge.

Research conducted in the lab has implicated the solute carrier family 46 (SLC46s) in muropeptide transport in flies and mammals. We have previously shown that one of the 8 *Drosophila* paralogs of the family, CG8046, is a key transporter of DAP-muropeptides in the Malpighian tubules of adult *Drosophila*, where it activates PGRP-LE. Publicly available microarray data have uncovered another SLC46, *CG15553*, as a potential candidate for muropeptide transport. *CG15553* was found to be expressed in the major immune organ, the fat body, and in the spermatheca, a female reproductive organ involved in sperm storage. Interestingly, *CG15553* expression changes with mating status, with *CG15553* being more highly expressed in virgin females. Moreover, flies treated with DAP-muropeptide further induced *CG15553* expression in the spermatheca and the carcass, hinting at an immune function. Mutants lacking *CG15553* showed decreased AMP gene induction in the spermatheca following local DAP-muropeptide challenge. In this study, we continue to investigate the role of the SLC46 family member *CG155553* in muropeptide transport and immunity.

224F ***drop-dead* mutants show altered cortex glial (CG) morphology and age-dependent hyperactivation of innate immunity (HII)** Unmila Jhuti, Edward BlumenthalDepartment of Biological Sciences, Marquette University

The involvement of glial cells has been demonstrated in several adult neurodegenerative diseases such as Parkinson's and Ataxia Telengiectasia. *Drosophila* models of neurodegeneration (ND) can be useful in such cases of ND where glial cells are involved. Hyperactivation of innate immunity (HII) has also been linked to ND both in humans and *Drosophila*. However, a specific role of glial cells and the involvement of HII pathways in ND have yet to be determined.

drop-dead (*drd*) is a gene with unknown function whose absence causes altered glial morphology and ND in adult fly brains. *drd* mutant brains start to show cell death right after eclosion and the flies die in the first week post-eclosion due to ND and other *drd* phenotypes. Although several mechanisms of cell death have been identified in mutant brains, the specific cell(s) that show ND was not distinctly identified. Immunostaining of individual glial cells using MCFO technique shows the presence of a subpopulation of altered cortex glial cells (CGs) in drd^{lwf} mutant brains which are around 10-15% of total CG population. These CGs are smaller in size and unlike regular CGs, they do not wrap around neighboring neurons. Such smaller CGs can be detected as early as the day of eclosion (P0). Other types of glial cells such as wrapping and ensheathing, perineurial, astrocyte-like, and subperineurial glia do not show such a phenotype. The apoptosis specific marker Dcp-1 was also detected around the altered CGs, which suggests that the presence of abnormal CGs may cause cell death in brain.

CGs provide support to neurons and act as a site for immune function in the brain. Thus, there could be a connection between CG alteration and HII in *drd* mutants. Preliminary data showed *drd* mutant flies that are heterozygous for mutations in the immune genes *relish* or *imd* live longer (median survival: 10-12 days) compared to the mutants with no immune genes mutation (median survival: 4-8 days). Preliminary data also suggest an age-dependent connection of HII with ND. Immunostaining showed increased expression of GFP reporters for several antimicrobial peptides (AMPs) in P2 mutant brains compared to controls. qPCR showed that the P2 and P4 flies show respectively 10 to 1000-fold upregulation of expression of the AMPs *diptericin*, *cecropin*, and *attacin* in mutant brains compared to control. The trend of HII upregulation is age-dependent where P4 flies show increased upregulation in mutants compared to P2 control brains.

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Keywords: cortex glia, innate immunity, cell death, aging, neurodegeneration.

225F **Not quite FedEx: How are venom proteins packaged for delivery by the parasitoid wasp *Ganaspis hookeri*?** Nicholas Bretz[1,2], Chris Lark[2], Nathan T Mortimer[1,21]Department of Biochemistry and Biophysics, Oregon State University, [2]School of Biological Sciences, Illinois State University

Parasitoid wasps are common pathogens of *Drosophila melanogaster*, and use venom proteins in order to overcome fly immunity. Venom proteins derived from the parasitoid wasp species *Ganaspis hookeri* alter the immune response mounted by immune cells known as plasmatocytes within infected *D. melanogaster* larvae. This venom activity is

mediated by a unique venom-specific isoform of the SERCA (Sarco/endoplasmic reticulum Ca^{2+}-ATPase) calcium pump. Venom SERCA activity inhibits the calcium burst normally mounted by plasmatocytes following infection, rendering them unable to melanize the foreign wasp egg. The mechanism by which SERCA and other venom proteins are transported into the host is not completely understood, but our preliminary evidence suggests that venom proteins are packaged into venom-specific vesicles known as venosomes. Ultracentrifugation of *G. hookeri* venom separates venom proteins into unique fractions. Nanoparticle tracking analysis and dynamic light scattering show the presence of vesicles in two of these fractions. Identification of protein content of these two fractions further supports the idea that venom proteins can be stored as cargo within venosomes. We hypothesize that venosomes allow venom proteins to gain access to plasmatocytes likely via the interaction between virulence factors present on venosome surface and host factors on the surface of plasmatocytes. In ongoing experiments, we are using vesicle imaging, mass spectrometry, SERCA activity assays, and spectrophotometric analyses to further characterize the putative venom vesicles.

226F **High dietary sugar post-development increases susceptibility to bacterial infection in *Drosophila melanogaster*** Andrea Mae Darby[1], Destiny O Okoro[1], Ashley M Frank[1], Brian P Lazzaro[1,2]1Entomology, Cornell University, [2]Cornell Institute of Host-Microbe Interactions and Disease, Cornell University

An organism's diet is a critical factor for surviving infection. Overnutrition with dietary sugar in particular can worsen infection outcomes in *Drosophila melanogaster* through unknown mechanisms. Prior studies were performed using flies that fed on high-sugar (HS) diets throughout larval development, thus making it difficult to distinguish pleiotropic developmental consequences from acute metabolic effects on immunity. We hypothesized that even transient exposure to HS diets may cause metabolic dysregulation with adverse consequence for immunity. To test this hypothesis, we reared *D. melanogaster* to pupation on a standard diet containing 4% (w/v) sucrose and 6% (w/v) yeast. At pupation, the rearing diet was replaced with one of six experimental diets varying from 0%-24% (w/v) sucrose and 6% (w/v) yeast. Newly eclosed adults fed on these diets 3-5 days prior to all assays. We found that post-eclosion exposure to HS diets caused elevated levels of glucose, glycogen, and trehalose despite these flies exhibiting reduced feeding rates. We assayed survival of systemic infection by four bacterial pathogens: two Gram-negative bacteria (*Providencia rettgeri* and *Serratia marcescens*) and two Gram-positive bacteria (*Enterococcus faecalis* and *Lactococcus lactis*). Flies given HS diets post-eclosion were more susceptible to infection by the Gram-negative bacteria, with those flies showing faster rates of *in vivo* bacterial proliferation and higher likelihood of death. Dietary sugar had much less effect on susceptibility to Gram-positive infection. We measured *in vivo* bacterial proliferation in flies that lack a functional immune response and found that *P. rettgeri* grew at the same rate regardless of diet whereas *S. marcescens* proliferated more rapidly in flies provided with the HS diet. Corroborating these results *in vitro*, we found that *P. rettgeri* is nitrogen-limited *vitro* whereas *S. marcescens* is more carbon-limited. Together, these data suggest that *Drosophila* fed on HS diets may be a nutritionally richer environment for *S. marcescens*, but that the susceptibility of HS-fed flies to *P. rettgeri* infection is likely due to immune impairment. To test the effect of HS diet on immune function, we measured expression of six genes encoding antimicrobial peptides (AMP), but found no difference across diets. We are currently testing whether HS diet impairs translation of AMP peptides. These data are foundational to understand the mechanisms by which high dietary sugar worsen infection outcome.

227F **Dietary macronutrients and live yeasts influence the microbiota composition of drosophila melanogaster** Ryan P Barney[1], John M Chaston[2]1Brigham Young University-Provo, [2]Plant & Wildlife Sciences, Brigham Young University-Provo

An often-underappreciated contributor to the overall health of an organism is its community of commensal microorganisms (microbiota). In *D. melanogaster*, the microbiota influences, for example, dietary preference, starvation resistance, and life-history strategy. Other studies have suggested an important relationship between the gut microbiota and a fly's ability to adapt to variation in macronutrient availability, but this is an under-explored area.

Also missing in the literature is a systematic investigation of the role of yeasts, important parts of the natural *Drosophila* microbiota, on the bacterial community of fruit flies. In this study we investigate both of these issues using a nutritional geometry approach. We inoculated flies with one of two microbial slurries—a 10-species bacterial mixture, or the same mixture supplemented with the yeast *S. cerevisiae*—and raised the flies on each of ten diets of varying protein:carbohydrate ratios. Then, we performed culture-independent (16S rRNA V4 marker gene sequencing) and culture-dependent analyses to define the microbial communities in flies on each diet and to define the differences in microbial community composition of flies reared on the different diets. Preliminary analyses have revealed that the fly microbiota differs in flies reared on the different diets or in the presence of yeast. The results provide insights

into possible consequences of seasonal changes in natural fly dietary substrates on the microbiota of wild flies, and consequently the microbially-influenced life history traits of the flies.

228S **Host feeding preferences shape microbiota composition in the fruit fly *Drosophila melanogaster*** Caroline Massey[1], Maggie Johnson[2], John M Chaston[21]Plant and Wildlife Sciences, Brigham Young University, [2]Brigham Young University

The microbiota plays an important role in the development of an organism and their life history traits, including fecundity and lifespan. Host genotype can significantly alter the microbiota composition in *Drosophila melanogaster*, meaning that host genotype can determine at least in part which microorganisms are present and how abundant they are. To understand the mechanisms by which host genotype selects the microbiota composition, I previously performed an assay where I measured the variation in the microbiota composition of different fly populations when they were given a choice of the two dominant types of bacteria in the *D. melanogaster* microbiota, Lactobacillus (LAB) and Acetobacter (AAB); or when no choice was provided. I found that flies from different geographies only have different microbiota compositions when they can choose. This geography specific feeding preference explains in part how host genotypes can select for different microbiota compositions. However, I also found that my assay design had a possible confounding factor: I inoculated the plates with equivalent CFU counts of AAB and LAB on fly diets. Unexpectedly, this resulted in ~ 50x more AAB on the plates 24 h later when the flies were introduced, meaning that the bacterial choices were not equivalent in abundance. It is not fully understood what the possible effects of starting with an unequal ratio of microbes might have had on my previous diet assay results. Therefore, I am continuing my work by modifying my protocol to increase the starting inoculum of LAB so that there is a 1:1 ratio of AAB and LAB on the diet at the time flies are exposed. Continuing this work will contribute to uncovering how big of an influence host genotype has on the microbiota variation observed between genetically distinct organisms.

229S **Genotypic effect on microbiome composition and colonization in *Drosophila melanogaster* models of Parkinson's disease** Shelby Olson[1], Samantha M Chagolla[1], Gerald B Call[21]Midwestern University, [2]Pharmacology, Midwestern University

Parkinson's disease (PD) is often associated with predominantly motor and neurological symptoms. Recently, the development of non-motor symptoms have begun to be associated with PD, with the most common symptoms being gastrointestinal (GI) disturbances such as gut dysbiosis and constipation. Due to the high prevalence of these GI symptoms, it is thought that there is a mechanism of communication between the gut and brain. Our laboratory has found that the microbiome has developmental effects in PD model flies. Another study by our laboratory found Acetobacter tropicalis present in 72% of various PD model flies, compared to 47% in control flies, indicating that there may be something important about A. tropicalis in PD model flies. To further pursue this, we inoculated both control and PD model flies with a combination of equal amounts of four different bacterial strains, including L. brevis, L. plantarum, A. pomorum, and A. tropicalis. Overall, there was a much higher level of total colonization in the homozygous park25 flies compared to all of the controls, and all flies that received the combination inoculation had an increased relative amount of A. tropicalis in their microbiome. This was most predominant in the park25 homozygous flies, where A. tropicalis accounted for over 90% of the total microbiome. While park25 flies have a large increase in the amount of bacterial colonization when compared to the control flies, the cause of this remains unknown although one suspicion is the presence of leaky gut syndrome (LGS). To test this, we performed a Smurf assay on both the control and PD flies, but the result did not indicate the presence of LGS. To determine the location of the bacterial overgrowth, the GI tract of control and PD model flies were dissected, separated into distinct sections, and plated. It was determined that the majority of the increased growth was occurring specifically in the crop, with all other sections showing no significant difference between PD and control flies. While there is strong evidence that the amount and composition of the microbiome is caused by the park25 allele, a trans-heterozygous PD model (park25/Df(3L)BSC553) was used to confirm our results. This trans-heterozygous model demonstrated a similar microbiome phenotype and also a decrease in climbing and flight ability when compared to the control flies, validating the results from the park25 fly.

230S **Effect of exogenous fecal exposure on protein aggregation and microbiome in a *Drosophila melanogaster* model of Parkinson's disease** Sharon Shaju, Regina Lamendella, Kathryn A JewettBiology, Juniata College

Parkinson's disease (PD) is a neurodegenerative disease involving increased protein aggregation, loss of motor skills, and memory loss. Studies on PD in humans also show great differences in the gut microbiome of affected and non-affected individuals. Some PD patients suffer from intestinal problems like constipation even before the onset of motor

symptoms. There are theories of PD progression supported by animal models that suggest abnormal protein aggregation starts in the gut and spreads to the central nervous system.

Disruptions in the *GBA* gene, encoding the lipid-modifying enzyme glucocerebrosidase, predispose humans to PD, and *Drosophila melanogaster* lacking this gene serve as a well-characterized model of PD. We used two fly genotypes, *GBA*-deletion, consisting of an almost complete deletion of the *Gba1b* gene (fly ortholog of human *GBA*), and a revertant control. *GBA*-deletion flies have increased protein aggregation, neurodegeneration, climbing deficits, and a shortened lifespan. Our lab has also observed significant differences in the dissected gut microbiomes of *GBA*-deletion flies. We were heavily interested in whether influencing changes in the gut microbiome would cause changes in other symptoms, specifically protein aggregation, so we focused on doing so through fecal exposure in pre-inoculated fly food vials.

We inoculated a vial containing fly food medium with 40 control flies, *GBA*-deletion flies, or no flies for 4 days, removed them, and then replaced them with 15 freshly eclosed *GBA*-deletion flies for an additional 3 days. After 3 days, the flies were then processed and analyzed through western blot for the protein Ref2P, a marker of protein aggregation, and in parallel, guts were dissected to run 16S rRNA Illumina MiSeq sequencing to determine how their gut microbiome may have changed. *GBA*-deletion flies have previously shown increased protein aggregation (Ref2P) compared to controls, and in preliminary studies we have seen differences in alpha diversity and enriched taxa between genotypes. We hypothesize that *GBA*-deletion flies exposed to the fecal matter of control flies through pre-inoculated food will have altered microbiomes and decreased protein aggregation, showing signs of bettering symptoms.

231S **Seasonal and diet effects on the lactic acid bacteria composition of *Drosophila melanogaster*** Joseph Gale, John Chaston, Reese Hunsaker, Aubrey Johansen, Amanda MorrisonPWS, Brigham Young University

The Drosophila microbiota can influence fly physiology, and natural variation in the abundance of each bacterial species can have consequent influence on host life history traits. Recent studies on the fruit fly microbiota have raised questions about the causes of natural geographic variation in the fruit fly microbiota, which have been hypothesized to result from variation in environmental factors and host genotype. In particular, one of our early studies identified lactic acid bacteria (LAB) as abundant in wild flies in certain locations; but follow up studies by us and others have been unable to replicate these early findings. We want to know how different environmental factors such as temperature, photoperiod, and humidity impact the LAB abundance in the Drosophila microbiome. To this end, we reared flies under different environmental conditions and quantified the relative bacterial abundances in them. Early findings suggest that of the three factors, temperature has a significant effect on the microbiota, and there is a higher LAB abundance at lower temperatures. We also seek a better understanding of how both time of season and degree of rottenness of the fruit affect LAB abundance. To answer this question, we continuously sampled flies off of 7 rotting piles of fruit set up at different time intervals to control for the time of the season. Ongoing sequencing and analysis will explore these roles further. This research will allow us to better understand why we are seeing changes in LAB abundance at different geographical locations.

232S **Characterizing Translational Shifts and Restricting Dietary Yeast During Bacterial Infection** Kate L Browning, Brian P LazzaroEntomology, Cornell University

A systemic bacterial infection induces a massive upregulation of immune genes, which then requires ribosomes to discern high-priority immune-related transcripts from background mRNA. In D. melanogaster larvae, this discrimination is mediated by 4E-BP (Thor), which enables selective translation of antimicrobial peptides (AMPs) through the use of internal-ribosome entry sites (IRES). D. melanogaster mutants for 4E-BP are highly susceptible to bacterial infection as adults. We hypothesize that translation of AMP-encoding transcripts becomes prioritized over homeostatic mRNAs in adult Drosophila, and that infection will lead to an upregulation of genes that can support the energetic demands of the immune-responsive cell. To better characterize changes in gene expression and translation priority, we are performing ribosomal footprinting and polysome profiling to test the hypothesis that immune-related transcripts gain translation priority during the early phase of infection. Additionally, the high translational demand of an immune response requires an ample supply of amino acids. These could be mobilized from internal stores or they could be acquired through active feeding. To begin to distinguish between these two possibilities, we tested resistance to infection in adult D. melanogaster that were reared through larval stages on a diet that contained 6% (w/v) dietary yeast (the major source of dietary protein), but then as adults were transferred to experimental diets containing 0%, 3%, or 6% yeast at either 3 days preceding or at the time of a bacterial infection. We delivered infection doses of either 250 or 5000 cfu/fly of a Gram-negative bacterium, Providencia rettgeri, and measured survival for 5 days post-infection. We found that regardless

of infection dose, Drosophila provided with 0% or 3% yeast diet prior to infection were highly significantly more likely to die from infection. However, when the flies were switched to the experimental diets at the time of infection, the flies on 0% yeast diets performed as well as flies on 6% yeast diets, and only the flies provided with 3% yeast exhibited excess mortality. These data suggest that protein stores at the onset of infection are critical for determining infection outcome. They also suggest that flies with adequate pre-infection stores but that are severely deprived of protein post-infection may engage a compensatory mechanism that allows them to survive infection. We are currently measuring the feeding rates and nutritional intakes of flies provided with each diet to test whether plasticity in feeding behavior affects protein-dependent resistance to infection.

233S **The Effects of Thiamine on Drosophila Dietary Yeast Preference** Dean Peterson[1], John Chaston[21]Life Sciences, Brigham Young University, [2]Brigham Young University

The microbiota of *Drosophila melanogaster* fruit flies can influence fly phenotypes. Here, we focus on the microbiota's effects on fruit fly dietary yeast preferences (DYP) to determine if specific nutritional molecules produced by the microbiota control DYP. Previous studies have ruled out that bacterial essential amino acids influence fly DYP, but we recently identified bacterial thiamine biosynthesis as a possible mechanism for bacteria to influence fly DYP. Here, we work to explicitly test if thiamine influences DYP by measuring fly DYP in flies reared on diets supplemented with thiamine. Then, we investigate the role of bacterial thiamine on these phenotypes by measuring DYP in flies colonized with bacterial thiamine biosynthesis mutants while reared on thiamine supplemented diets. We expect that if bacterial thiamine production influences fly DYP, then flies raised with increased thiamine in their diet will have diminished preference for dietary yeast. Together, these approaches will help to explain genetic mechanisms by which bacteria influence the feeding preferences of their hosts.

234S **Vertically Transmitted Avirulent Nora Virus is Associated With Canonical Toll and Imd Pathway Gene Expression in Cells of the Fat Body** Robert L Unckless, Nilanjan RoyMolecular Biosciences, University of Kansas

Viruses are ubiquitous and can spread vertically through the embryo or in early-stage larvae. If virulence is low, these viruses can go undetected and can build up persistent infections. In many *Drosophila* studies, researchers are unaware or ambivalent about the fact that the flies may be infected with vertically transmitted avirulent viruses, and these viruses can affect the canonical gene regulation systems in *Drosophila*. In this study, we show the vertically transmitted avirulent Nora virus, found in a published *Drosophila* fat body single-nuclear RNA sequencing (snRNA) experiment can influence the innate immune system. The original experiment infected virgin or mated female flies with or without the bacteria *Providencia rettgeri* leading to four different treatment conditions: virgin uninfected (VU), virgin infected (VI), mated uninfected (MU), and mated infected (MI). We found that these individuals were infected with both *Drosophila* Nora Virus and *Drosophila* A Virus. As the Nora virus mRNA was detected in all four treatment conditions, we restricted this study to the Nora virus to learn about how it impacts the immune system. There were substantially more Nora virus-infected cells in samples *not* infected with *P. rettgeri* (22.83% of total fat body cells) compared to those infected (9.49% of total fat body cells). To determine the immune genes that are most associated with viral infection in individual cells, we performed linear regression analysis comparing normalized gene expression per cell with normalized viral RNA read count. Our regression analysis showed that the expression pattern of *Drosomycin* (Drs), which is regulated by the Toll (mostly) and Immune Deficiency (Imd) pathways, is strongly correlated with Nora virus RNA copy number with a correlation coefficient of 0.83. Additionally, we observed correlation between the Nora virus and the genes *spirit, IM23, IM1, and SPE* of the Toll pathway, as well as *pirk and scny* of the Imd pathway ($P<0.001$ and correlation coefficient of 0.30 or greater). *AGO2* expression (a component of the RNAi pathway which is established for antiviral response in *Drosophila*) showed around 21% correlation with Nora virus as well. The genes of JAK-STAT immune signaling pathway showed no association with the Nora virus RNA level. Using a similar method, we also searched for Nora virus associations with transmembrane proteins to identify potential virus entry receptors. Overall, our research shows that undetected avirulent viruses like the Nora virus in flies may trigger specific immune genes in the Toll, Imd, and RNAi pathways in the fat body of *Drosophila*. These results lead up to questions such as whether Nora virus targets cells with higher levels of baseline immune gene expression or Nora-virus infection in individual cells induces these responses. Our future work will attempt to disentangle these possibilities.

235S **Renal NF-κB activation impairs uric acid homeostasis to shorten lifespan in the context of malignant tumors** Yuchen Chen, Wei SongWuhan University

Tumor-induced host wasting and mortality are general phenomena across species. Many groups have previously

demonstrated endocrinal impacts of malignant tumors on host wasting in rodents and Drosophila. Whether and how environmental factors and host immune response contribute to tumor-associated host wasting and survival, however, are largely unknown. Here, we report that flies bearing malignant yki^{3SA}-gut tumors exhibited the exponential increase of commensal bacteria, which were mostly acquired from the environment, and systemic IMD-NF-κB activation due to suppression of a gut antibacterial amidase PGRP-SC2. Either gut microbial elimination or specific IMD-NF-κB blockade in the renal-like Malpighian tubules potently improved mortality of yki^{3SA}-tumor-bearing flies in a manner independent of host wasting. We further indicate that renal IMD-NF-κB activation caused uric acid (UA) overload to reduce survival of tumor-bearing flies. Therefore, our results uncover a fundamental mechanism whereby gut commensal dysbiosis, renal immune activation, and UA imbalance potentiate tumor-associated host death.

236S **The conserved acetyltransferase Tip60 modulates insulin signaling and lipid homeostasis in *Drosophila melanogaster*** Juliana H Batista, Paula WatnickInfectious diseases, Boston Children's Hospital/Harvard Medical School

The Tip60 complex is a well-studied regulator of cell homeostasis, the stress response, cell death, and stem cell division. It is best known as an activator of transcription and is implicated in the regulation of key tumor suppressors and oncogenes such as p53 and Myc. In *Drosophila*, the Tip60 complex was previously associated in the IMD signaling, and our lab found more recently that in response to acetate production by intestinal microbiota, the Tip60 complex in Tk-expressing enteroendocrine cells activates the intestinal innate immune response and represses lipid synthesis. Tip60 is expressed in all intestinal cell types. To identify a microbiota-specific role for Tip60 in enterocytes, we knocked down Tip60 using a Myo1A driver (NP1>Tip60^{RNAi}) and characterized the phenotype of these flies. We found that NP1>Tip60^{RNAi} flies were delayed in development and smaller than the driver-only controls. We hypothesized that the difference in size could be due to a metabolic impairment. In agreement with our hypothesis, reduced expression of Tip60 in enterocytes resulted in decreased lipid stores and glucose levels, suggesting a metabolic imbalance. Furthermore, western blot analysis of AKT showed increased levels of phosphorylation in NP1>Tip60^{RNAi} flies as compared with controls. We hypothesize that the Tip60 complex in enterocytes is essential to maintain a level of insulin signaling that supports normal fly development and adult metabolic homeostasis.

237S **Evolution of mutualism from parasitism in a *Wolbachia-Drosophila* symbiosis** Jessamyn I Perlmutter, Robert L UncklessMolecular Biosciences, University of Kansas

Animal-microbe symbioses are ubiquitous in nature, spanning diverse interactions from facultative parasitism to obligate mutualism. Further, many of these relationships are not strictly parasitic or mutualistic, and there can be an evolutionary continuum between the two states. Indeed, many facultative symbionts co-evolve with their hosts and develop obligate relationships. However, the transition between symbiotic states is rarely observed and little is known about the genetics and mechanisms underlying symbiotic transition points. Here, we report the discovery of a male-killing symbiont that has developed mutualistic traits. Male killing, or selective death of male offspring, is a phenotype that can result when certain strains of *Wolbachia* bacteria infect their arthropod hosts. We discovered a lineage of *Drosophila bifasciata* that exhibits developmental and fitness defects when its male-killing *Wolbachia* symbiont, *w*Bif, is cured via heat or antibiotic treatment. In contrast, a fly lineage that has never carried the symbiont exhibits no defects with treatment. These differences are uncharacteristic of hosts infected with male-killing *Wolbachia*, and it is previously unobserved even in other *w*Bif-*D. bifasciata* lineages. These results suggest that this is a potentially recent and evolving mutualism where the infected fly lineage has acquired a developmental defect that rescued by *w*Bif. In addition, the mitochondria of the infected lineage are highly divergent from other *D. bifasciata* lines, and evidence suggests they may be the source of the host developmental defect. This *Wolbachia-Drosophila* symbiosis represents a potential new case of a symbiosis in transition to mutualism, and allows rare insight into the molecular changes underlying this phenomenon.

238V **A novel interaction between an intracellular pathogen effector protein and the host Hippo signaling pathway** George Aranjuez, Travis J JewettImmunity and Pathogenesis, University of Central Florida

Chlamydia trachomatis infection is the most frequently reported sexually transmitted infection in the United States. As an obligate intracellular bacterial pathogen, *Chlamydia* injects multiple protein effectors via the type III secretion system (T3SS) into the host cell to induce its entry and establish a replicative niche. The early effector Tarp is required for efficient host cell entry by *Chlamydia* via the ability to polymerize and bundle host F-actin mediated through Tarp's C-terminal region. In contrast, not much is known about the function of Tarp's N-terminal region (N-Tarp) in the host cell. The function of a single effector domain is difficult to resolve amidst all the host cell responses in an in vitro infection model. To address this, we utilize *Drosophila* as an in vivo cell biology platform to study effector-host interactions.

Drosophila development is well-characterized down to the molecular level such that developmental phenotypes can be traced back to the perturbed molecular pathway. Transgenic expression of N-Tarp throughout the whole animal results in complete lethality. Restricted expression to the dorsal thorax results in surviving flies with abnormal duplication of mechanosensory bristles, previously observed in flies with altered Hippo signaling. The Salvador-Warts-Hippo pathway is a conserved signaling cascade that regulates host cell proliferation and survival during normal animal development and in some forms of cancer. To explore this further, we expressed N-Tarp in actively growing larval imaginal wing discs, which are sensitive to perturbations in Hippo signaling. N-Tarp expression results in larval wing disc overgrowth with concomitant increase in adult wing size. N-Tarp-induced overgrowth phenocopies perturbations in Hippo signaling that lead to increased proliferation. Last, N-Tarp expression results in altered expression of Hippo pathway target genes. Ongoing genetic interaction experiments will pinpoint the exact mechanism of action of N-Tarp in the context of Hippo signaling. Thus, we provide the first direct evidence that the N-terminal region of the *Chlamydia* effector Tarp can alter the host Hippo signaling. Altered Hippo signaling might inhibit apoptosis of an infected host cell, allowing *Chlamydia* development to complete.

239V **No blood, no guts, no glory: the gut-immune axis during injury** Sveta Chakrabarti, Sandhya VisweswariahIndian Institute of Science

We showed previously a dramatic upregulation of the JAK/STAT cytokine upd-3 (IL-6 like) and the Toll signalling pathway post-injury. We showed that intracellular accumulation of hydrogen peroxide in hemocytes is critical for upd-3 induction. How does hydrogen peroxide gets imported into hemocytes? We revealed that the diffusion of hydrogen peroxide occurs with the help of a channel protein Prip. And, in the absence of Prip, cytokine upd-3 production is impaired. Strikingly, injured flies are better protected from infection with a bacterium like *Enterococcus faecalis,* as injury-based hemocyte activation can train the immune response for subsequent infection (PMID: 33176146).

The gut is associated with approximately 70% of all immune cells present in the body in humans. The proper function of the gut is imperative for the appropriate uptake of nutrients from food, without which there are adverse effects to the entire body. My earlier work showed that the blood cells of flies act as a messenger between the distant site of wounding and the intestine to regulate stem cell proliferation. If blood cells are not activated post-injury without a hydrogen peroxide signal, then the intestinal stem cells fail to proliferate, and this causes increased susceptibility to the injury. I am currently working out the molecular mechanisms of how intestinal stem cell proliferation is triggered post an injury to the thorax in flies. This happens to the breakdown of the gut barrier function due to improper turnover of the enterocytes that require STAT transcription faction activation. I will present the inter-organ communication between hemocytes and the gut upon injury, where I explore the role of blood cells in host physiology and health post-wounding. In this study, we show that hemocytes home to the gut following a distal injury and play an essential role in survival to a subsequent infections with enteric pathogens.

240V **Defining the Role of Cyclic Dinucleotide (CDN) Induced Genes in Innate Antiviral Immunity in Drosophila** Steven Miller[1], Elisha Segrist[2], Beth Gold[3], Jesse Hulahan[3], Marie Arvidson[3], Sara Cherry[31]Microbiology, University of Pennsylvania, [2]National Institutes of Health, [3]University of Pennsylvania

The gut microbiota plays an important role in maintaining overall health and immunity while the STING pathway plays an important role in immune protection. STING is activated by Cyclic dinucleotides (CDNs) to induce antimicrobial gene expression dependent on NF-kB signaling. CDNs can be generated by two sources, first endogenously by Cyclic-GMP-AMP synthase (cGAS) in vertebrates and cGAS-like receptors (cGLRs) in invertebrates. A second source of CDNs are from the microbiota, which can also be sensed by STING. We found that bacterial derived CDNs prime antiviral immunity in the Drosophila intestine and set out to define the gene expression program regulated by this pathway in the gut. We profiled the intestine from wild type flies as well as from STING and NFKB mutants stimulated by oral feeding of CDNs. We identified 32 genes that are induced by CDN feeding. Moreover, 24 of these genes were STING- and NF-kB-dependent (17 of which are conserved in mammals) and included the previously characterized Srg1-3. We are performing genetic studies to define the roles of these genes in antiviral immunity during both oral and systemic infections. This study will increase our understanding of the genes and pathways downstream of the STING pathway that are involved in protection from virus infection.

241V **Does varying investment in egg production modify immune defense in mated female *Drosophila melanogaster*?** Kathleen E Gordon[1], Shravasti Ray[1], Patrick Gonzales[2], Mona Li[2], Crystal Liang[2], Mariana F Wolfner[2], Brian P Lazzaro[1,31]Entomology, Cornell University, [2]Molecular Biology and Genetics, Cornell University, [3]Cornell Institute of Host

Microbe Interactions and Disease, Cornell University

In *D. melanogaster* and many other species, female reproductive investment comes at a cost to immunity and resistance to infection. During mating, the male transfers seminal fluid proteins like Sex Peptide (SP) that reduce female immune capacity. At least some of this effect may be due to SP-mediated stimulation of egg production, as female flies without a germline, and who therefore produce no eggs, retain high immune capacity upon mating. Because maturation of eggs requires abundant production of yolk proteins (YPs) in the fat body, a tissue that also produces antimicrobial peptides (AMPs) in response to infection, we are testing whether the level of investment in YP production might directly or indirectly trade off with immune capacity. First, we are testing fertility and immune phenotypes of unmated versus mated females with missense mutants in the three YP genes (Tanaka et al. 2021) and with YP null mutations that we are generating with CRISPR-Cas9 genome editing. Second, we are testing whether females with early oogenesis defects (*egaliatarian, bam*, *ovoD, yolkless*) invest fewer resources, such as YPs, into reproduction, allowing for the maintenance of immune capacity after mating. In parallel we are testing sterile females with late oogenesis defects (*okra, retained)*, as we expect that these would still invest resources into egg production and therefore continue to suffer from reduced immune capacity after mating. Consistent with these hypotheses, we found that mating reduced the immune capacity of late-arresting *okra* and *retained* mutant females, while early-arresting *egalitarian* mutant females maintained immune capacity after mating. Interestingly, *yolkless* mutant females, who make YPs but cannot incorporate them into oocytes, continued to suffer reduced immune capacity after mating. Although *yolkless* is an early-arrest mutant, females still appear to pay the cost of YP production, consistent with our hypothesis that YP production in the fat body limits mated female immune capacity. Together, these results can connect how abundant egg and YP production, triggered by mating, interferes with the immune response within the fat body, resulting in a physiological trade-off between reproduction and immunity.

242V **PTTH regulates lifespan through innate immunity pathway in *Drosophila melanogaster.*** Ping Kanglowa State University

The prothoracicotropic hormone (Ptth) is well-known for its important 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) show extended lifespan and healthspan, and exhibit increased resistance to oxidative stress. Transcriptomic analysis reveals that age-dependent upregulation of 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 overexpression of Relish shortens the lifespan, while oenocyte-specific downregulation of ecdysone signaling extends lifespan. Consistently, knocking down torso, the receptor of Ptth in the prothoracic gland also promotes longevity of the flies. Thus, our data reveal a novel function of the insect hormone Ptth in longevity regulation and innate immunity in adult *Drosophila*.

243V **The influence of environmental variation on the dynamics of host-symbiont interactions between *Drosophila melanogaster* and *Wolbachia*** Anton Strunov[1], Wolfgang Miller[1], Martin Kapun[2,31]Dept Cell & Developmental Biology, Medical University Vienn, [2]Department of Cell & Developmental Biology, Medical University Vienn, [3]Central Research Laboratories, Natural History Museum Vienna

Endosymbiotic microbes compete with their host for resources. However, they may also provide beneficial metabolic activity that improves the host fitness. The balance between mutualistic and parasitic interactions may be strongly influenced by environmental variation which can result in titer variation of the microbe or affect the immunity of the host.

Host-symbiont interactions between *Wolbachia* and *Drosophila melanogaster* provide an excellent model to study this question, given that *Drosophila* occurs in a broad range of different environments and is commonly infected by mainly two different *Wolbachia* strains. One of these *Wolbachia* types, *w*MelCS, was only recently replaced by the *w*Mel variant in most world-wide populations of *D. melanogaster* flies, but the biological reasons for this turnover remain largely unknown.

In our work we tackle this unresolved question by investigating the influence of different *Wolbachia* strains on host fitness in different temperature regimes using naturally infected *D. melanogaster* strains from Portugal. We find that *Wolbachia* strains vary in titer levels at different temperatures and uncover complex temperature- and strain-specific effects of *Wolbachia* infections on fitness-related traits of their *Drosophila* hosts. Our findings thus indicate that host-

symbiont interactions in this system strongly depend on host and symbiont genotypes and on thermal conditions.

244V **Binding and Beyond: Exploring *Wolbachia's* Ankyrin Effector Proteins and their Impact on the Host Organism** Will Hamilton[1], Jon Massey[1], Erin Hardy[1], Irene Newton[21]Biology, Indiana University, [2]Biology, Indiana Univserity

Host organisms and the pathogenic microbes they encounter interact with each other in an arms race to benefit their own fitness. *Drosophila melanogaster* uses innate immunity to counteract invading microbial pathogens; however pathogens have evolved mechanisms by which to evade the host immune response. One such mechanism is the secretion of microbial proteins into the host cell, affecting the cell in a way conducive to the microbe's survival. In addition to immune system subversion, effector proteins can aid in invasion of specific host tissue or producing abnormal phenotypes in the host. My work aims to investigate the effector proteins produced by the alpha-proteobacterium *Wolbachia pipientis* in its natural host, *Drosophila melanogaster. Wolbachia* infects about 60% of all insect species on Earth, including other arthropods and filarial nematodes. These infections can induce severe host phenotypes, such as cytoplasmic incompatibility and male-killing, which dramatically change host reproductive fitness. How *Wolbachia's* effector proteins aid in the invasion, persistence inside, and interaction with host cells to cause such phenotypes remains largely unknown. The difficulty to understand this host-microbe relationship largely stems from *Wolbachia* being an obligate intracellular bacterium, meaning that it cannot be cultured outside of a host cell. Additionally, we do not currently have genetic tools to manipulate *Wolbachia*. To circumvent these obstacles and test how putative *Wolbachia* effectors impact host cell fitness I over-expressed effectors containing the ankyrin repeat motif, with the hypothesis that this highly conserved protein-protein interaction domain may mediate *Wolbachia*-host interaction. Of the 25 ankyrin-containing effectors, I discovered two which produced severe phenotypes in *Drosophila*. I then identified protein binding partners from the host by performing a genome-wide yeast-two-hybrid assay. Screening 10,168 Drosophila proteins, I discovered a single binding partner for each phenotype producing effector. My future work will focus on verifying these binding partners in vivo and unraveling the mechanisms by which these interactions occur.

245V **Effect of caffeine intake on *Drosophila*'s gut microbial community, innate immunity and susceptibility to pathogens** Abeer Qush[1], Hend Al-Tamimi[2], Alyaa Al-eshaq[2], Hajar Rachid[1], Shaima Al-adwi[1], LAYLA KAMAREDDINE[1,21]Biomedical Science, Qatar University, [2]Biomedical Research Center, Qatar University

Caffeine (1,3,7-trimethyxantine), which is a typical accompaniment in many types of sustenance including energy drinks, chocolate, soda, tea, and coffee, is known for being a psychotropic substance with central nervous system and metabolic provoking properties. This psychoactive ability of caffeine has been attributed to its intake dosage. While an intake of 100 mg/day could reduce exhaustion and weariness, elevate physical strength and energy, and positively trigger cognitive functions, higher caffeine concentrations of 400 mg/day and above subjects the consumer to a toxic status with episodes of vomiting and abdominal cramps, as well as to a risk of developing rhabdomyolysis. So far, most studies have focused on the effect of caffeine on mental processes, liver fibrosis, tumor cells, as well as on several physiological systems including the respiratory and the cardiovascular. Currently, an emerging body of evidence also supports an immunomodulatory effect of caffeine; yet, our understanding of the nature of this immune modulation and the mechanism of action employed herein remains at its infancy. In this study, we shed more light on this, with particular focus on the mechanistic effect of dose-dependent caffeine intake on gut microbiota, innate immunity, and host susceptibility to pathogens using the *Drosophila melanogaster* model organism.

246V **Time of day variation in gut microbes of *Drosophila melanogaster*** C. Isaiah Fitzmaurice[1], Teja Bhimavarapu[1], Alexis M. Burns[1], Paul M. Schweiger[2], Alder M. Yu[11]Biology, University of Wisconsin - La Crosse, [2]Microbiology, University of Wisconsin - La Crosse

The fly gut microbiome serves important functions in development, digestive function, and immunity, and may affect behavior via the gut-brain axis. In mammals, the taxonomic composition of the gut microbiome exhibits daily oscillations that interact with the circadian rhythms of the host via bidirectional signaling pathways. The objective of this research is to determine whether the taxonomic composition of the gut microbiome of *Drosophila melanogaster* shows time of day based variation, and whether any such variation depends on intact circadian rhythms in the host. Guts were dissected from surface sterilized flies and isolated DNA subjected to 16S Illumina sequencing. Amplicons were identified to the genus level via the EZBioCloud pipeline.

Wild-type (w^{1118}) flies entrained under 12:12 light:dark conditions showed robust time of day based variation in gut microbe composition. These variations persisted in constant darkness, but were abolished when flies were kept under

constant light conditions, suggesting that the variation may be due to the circadian clock function of the host. However, time of day based variations in gut microbe composition persisted under constant dark conditions in *per*^{01} mutant flies, which lack endogenous circadian rhythms. Similar preliminary results were obtained for *timeless* mutant flies. This suggests that rhythms in fly gut microbiota composition may not be entirely a response to host circadian clock signaling or behavioral rhythms.

247T **Rapid evolutionary diversification of the *flamenco* locus across simulans clade *Drosophila* species** Sarah Signor[1], Jeffrey Vedanayagam[2], Filip Wierzbicki[3], Robert Kofler[3], Eric C. Lai[21]North Dakota State University, [2]Sloan-Kettering Institute, [3]Vetmeduni Vienna

Effective suppression of transposable elements (TEs) is paramount to maintain genomic integrity and organismal fitness. In *D. melanogaster*, *flamenco* is a master suppressor of TEs, preventing their movement from somatic ovarian support cells to the germline. It is transcribed by Pol II as a long (100s of kb), single-stranded, primary transcript, that is metabolized into 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. *D. 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 multicopy elements, 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.

248T **Fast evolution of proteins involved in heterochromatin functions** Leila Lin[1], Yuheng Huang[1], Jennifer McIntyre[1], Ching-Ho Chang[2], Serafin Colmenares[3], Chwen G Lee[11]Ecology & Evolution, UC Irvine, [2]Fred Hutchinson Cancer Research Caner research center, [3]Department of Cell and Molecular Biology, UC Berkeley

The eukaryotic genome consists of two distinct compartments-the gene-rich, transcriptionally active euchromatin the gene-poor, transcriptionally inert heterochromatin. Despite the seeming lack of transcription activity in heterochromatin, it is known to play an important role in genome stability, chromosome segregation, and suppression of transposable elements. Decades of research have identified genes that enhance or weaken heterochromatin functions, known as Su(var)s and E(var)s respectively. In addition, genes whose protein products localize to heterochromatin are also increasingly identified to play an important role in heterochromatin functions. Because of the highly essential aforementioned functions of heterochromatin, it is expected that genes important for heterochromatin functions should be conserved. Yet, a handful of studies have identified that a few genes involved in heterochromatin functions evolve fast. Yet, it is still unclear whether the observed adaptive evolution is also applicable to other genes that encode proteins that modulate heterochromatin function and what the possible driving force is.

To address these questions, we investigated the evolution of genes that are involved in heterochromatin functions in Drosophila over both a short (~5 MYR) and long (~40 MYR) evolutionary time scale. We found that, compared to both genome-wide averages and genes involved in the regulation of other types of repressive chromatin (polycomb), genes involved in heterochromatin functions show exceptionally prevalent adaptive evolution over both evolutionary time scales. The rapid evolution of genes involved in heterochromatin functions is mainly in the form of changes in amino acid sequences, instead of protein copy number, which differs from previous observations made from few genes. Interestingly, many of the fast-evolving sites fall within nucleic acid binding domains, suggesting a possibility that the fast evolution of these genes is driven by their interactions with the rapidly changing heterochromatic repeats. Indeed, for several genes involved in heterochromatin function, we found their rates of protein evolution significantly correlate with the abundance of simple repeats across the phylogenetic tree. Our results suggested the prevalent adaptive evolution of genes involved in heterochromatin functions that is driven by the fast-changing repeatome.

249T **Twenty-seven ZAD-ZNF genes of *Drosophila melanogaster* are orthologous to the embryo polarity**

determining mosquito gene *cucoid* Muzi Li[1], Koray Kasan[1], Zinnia Saha[1], Yoseop Yoon[2], Urs Schmidt-Ott[11]The University of Chicago, [2]University of California, Irvine

The C2H2 zinc finger gene *cucoid* establishes anterior-posterior (AP) polarity in the early embryo of culicine mosquitoes. This gene is unrelated to genes that establish embryo polarity in other fly species (Diptera), such as the homeobox gene *bicoid*, which serves this function in the traditional model organism *Drosophila melanogaster*. The *cucoid* gene is a conserved single copy gene across lower dipterans but nothing is known about its function in other species, and its evolution in higher dipterans, including Drosophila, is unresolved. We found that *cucoid* is a member of the ZAD-containing C2H2 zinc finger (ZAD-ZNF) gene family and is orthologous to 27 of the 91 members of this family in *D. melanogaster,* including *M1BP, ranshi, ouib, nom, zaf1, odj, Nnk, trem, Zif,* and eighteen uncharacterized genes. Available knowledge of the functions of *cucoid* orthologs in *Drosophila melanogaster* suggest that the progenitor of this lineage specific expansion may have played a role in regulating chromatin. We also describe many aspects of the gene duplication history of *cucoid* in the brachyceran lineage of *D. melanogaster*, thereby providing a framework for predicting potential redundancies among these genes in *D. melanogaster*.

250T **Extreme QTL Mapping Reveals Zinc Resistance Loci** Katherine Hanson[1], Anthony D Long[2], Stuart J Macdonald[11]Molecular Biosciences, University of Kansas, [2]Ecology and Evolutionary Biology, University of California

Many heavy metals such as zinc, copper and manganese are essential for cellular function and maintaining metal homeostasis is critical. When exposed to toxic levels of heavy metals, including essential metals, organisms can suffer deleterious consequences, including increased risk for cancer and organ failure. Zinc is involved in many cellular functions, and zinc toxicity can have widespread effects, leading to necrosis, inhibition of mitochondria, and impacting the homeostasis of other heavy metals. The response to zinc toxicity is a complex, polygenic, trait, and our goal is to identify those genes segregating for allelic variation for zinc resistance using an unbiased genome wide mapping approach. *Drosophila melanogaster* is an ideal model to study zinc resistance since it possesses orthologs of critical genes involved in zinc homeostasis, such as *MTF-1* (a transcription factor involved in metal response), numerous zinc transporter proteins and has proved to be a successful model to understand a range of heavy metal response traits. To identify zinc resistance loci we employed extreme QTL, or XQTL, mapping, a powerful technique that identifies QTL via selecting and sequencing pools of outbred individuals with extreme phenotypes. Our population was established by mixing hundreds of DSPR (*Drosophila* Synthetic Population Resource) strains, a set of advanced intercross lines derived from 8 inbred founder lines. We raised animals from this population on media supplemented with toxic levels of $ZnCl_2$, sequencing 12 replicate pools of surviving, zinc resistant adult females, and a matching control population. We estimated the founder composition from each pooled sample, and at each position along the genome identified QTL as significant frequency shifts between control and selected populations. We identified 7 QTL, and for most only 1 or 2 founder alleles show a substantial frequency change between the control and selected populations, implying that highly resistant/susceptible alleles are often rare. One of the QTL overlaps with a locus previously implicated in copper developmental viability, potentially suggesting shared genetic control. We resolved 24 strong candidate genes within mapped zinc resistance QTL, and for effects on emergence and development via midgut-specific RNAi. Genes showing effects following RNAi include *MTF-1*, *Xrp-1* and *pHCl-2*. Our work highlights several recognized, and novel contributors to metal metabolism in flies.

251T **Molecular evolution in Oskar protein function in *Drosophila*** Emily L Rivard[1], John R Srouji[1], Cassandra G Extavour[1,2,31]Department of Molecular and Cellular Biology, Harvard University, [2]Department of Organismic and Evolutionary Biology, Harvard University, [3]Howard Hughes Medical Institute

Understanding how evolution in gene sequences leads to changes in the development of different species requires investigation of how the proteins encoded by these genes have functionally diverged. However, most studies of the genetic basis of evolutionary change have focused on *cis*-regulatory changes. We argue that characterizing protein evolution at a molecular level is essential to understand the evolution of binding interactions, enzymatic activity, or other aspects of protein function. This information is required to provide the link between sequence changes and corresponding effects on cellular and developmental phenotypes. To address this problem, we are interrogating how sequence changes in *oskar,* an insect-specific gene required for germ cell specification and embryonic patterning in fruit flies, have led to a previously observed functional incompatibility between *Drosophila melanogaster* and *Drosophila virilis*. We have generated sixteen chimeric versions of *oskar* that mix sequences of Oskar protein's functional domains from each species in several combinations. We are expressing these chimeric *oskar* sequences in *D. melanogaster* flies with and without endogenous *oskar* present and then characterizing each chimera's ability to perform *oskar* function.

More specifically, we are testing whether the chimeric Oskar proteins are capable of performing *D. melanogaster* Oskar's most well-characterized role of recruiting germ line determining and abdominal patterning molecules to the posterior end of the developing oocyte and early embryo. We are also testing whether the chimeric Oskar proteins can induce primordial germ cell specification and enable proper axial patterning in first instar larvae. We look forward to presenting the results of these ongoing experiments at the meeting. This study of *oskar* can provide valuable *in vivo* evidence of the specific molecular action of Oskar's functional domains. In addition, it will increase our understanding of how sequence changes translate to functional evolution and highlight the importance of post-transcriptional regulation mechanisms in key developmental processes.

252T **Single-cell analysis of *doublesex*-expressing neurons across species** Justin Walsh, Ian Junker, Yun DingUniversity of Pennsylvania

Understanding the evolution of complex traits, including behavior, is a main goal of evolutionary biology. Behavioral diversity across species is encoded by evolutionary changes in a neuron's morphology and function - a product of its molecular makeup. Therefore, comparing gene expression differences in homologous neurons between species is a powerful approach to identify genes that contribute to the evolution of neural differences underlying behavioral diversity. *Drosophila* courtship behavior displays a wide range of diversity across species (e.g. male courtship song). The majority of the neural circuitry underlying courtship behavior expresses the sex determination gene *doublesex* (*dsx*), including the neurons necessary for the production of courtship song. In both *D. melanogaster* and *D. yakuba*, we genetically labeled dsx-expressing neurons, isolated them, and performed single-cell RNA sequencing. Although the two species' neurons largely overlap in Uniform Manifold Approximation and Projection (UMAP) space, we found species-specific UMAP clusters, and all clusters demonstrate species-specific or -biased gene expression. Many of these differentially expressed genes have known neural or behavioral functions. Finally, we identified molecular markers for each UMAP cluster, allowing us to test the function of each cluster in the future, including species-specific clusters. Our study offers a novel perspective on how evolution has shaped gene expression patterns and neuronal numbers to result in functional adaptation across species.

253T **Genomic diversity reveals invasion history and environmental adaptation of *Drosophila suzukii*** Siyuan Feng[1], Samuel DeGrey[2], Sean Schoville[2], John Pool[11]Department of Genetics, University of Wisconsin-Madison, [2]Department of Entomology, University of Wisconsin-Madison

Biological invasions are of great research interest not only because they often carry significant economic and ecological costs, but also because they constitute natural experiments that allow investigations of evolutionary processes on contemporary timescales. The fruit pest *Drosophila suzukii*, which has rapidly invaded diverse environments in Europe and the Americas from Asia within the past few decades, stands out as an excellent model for studying invasion genomics and local adaptation. However, despite the recent availability of genomic resources in *D. suzukii*, the invasion routes have only been inferred from insufficient number of markers. Here, we investigated genomic diversity, invasion history and environmental adaptation of *D. suzukii* using whole-genome sequencing data and environmental metadata of 29 population samples from its native and invasive ranges of major occurrences. Strong founder event bottlenecks were suggested by the acute drop in nucleotide diversity within the invasive populations relative to the native Chinese and Japanese populations. Principal component analysis of allele frequencies and matrices of window F_{ST} and D_{XY} recapitulated the expected clustering of populations into three distinct ranges: Native, European, and American. All other populations were found to have a subset of the genetic diversity present in a sample from southeastern China (Ningbo), consistent with an ancestral or refugial species range in this region of Asia. We also found strong support for separate Asia-sourced invasion events into the Americas and Europe, and gene flows between them following the first founder events from tree-based population structure and admixture inferences. Using an F_{ST}-based genotype-environment association method, we detected signals of positive selection that are driven by distinct environmental factors including altitude, wind speed, precipitation, and human land usage. Gene overlap and functional enrichment analyses suggested a shared genetic and functional basis of local adaptation to related types of environmental pressure, which were partly driven by synaptic transmission genes, as well as functions specific to each environmental variable. We are also expanding such environment-associated genome scans to adaptive signatures of copy number variations and inversions. Our findings provide insights into the population history of *D. suzukii* and depict a finer-scale adaptive landscape underlying this species' invasion success.

254T **Centromere polymorphisms in *Drosophila melanogaster*** Miraz A Sadi, Cécile Courret, Amanda M LarracuenteDepartment of Biology, 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*. We have recently shown that in *D. melanogaster*, centromeres correspond to islands of retroelements flanked by simple tandem satellite repeats. To ask if those centromere islands are conserved within *D. melanogaster*, we used CUT&Tag to identify CENP-A-enriched DNA in individuals from populations around the globe. We identified the centromere islands, compared their organization and composition amongst the different populations, and validated our observations with FISH on larval brains using oligopaints specific to each centromere island. We tested individuals from 5 populations with different geographical origins and compared them to the reference strain *Iso1*. Our analyses reveal that both the dot and the X chromosome centromeres are polymorphic between individuals, the reference centromere islands are absent from the genome. Altogether, our results highlight that the DNA sequences underlying centromeres are plastic and can be highly dynamic within species.

255T **Functional evolution of horizontally transferred bacterial cytotoxins in a novel *Drosophila* innate immune module** Rebecca L Tarnopol[1], Jaden Ha[2], Josephine A Tamsil[3], Kirsten I Verster[4,5], Gyöngyi Cinege[6], Éva Kurucz[6], Zoltán Lipinszki[7], Susan L Bernstein[3], István Andó[6], Noah K Whiteman[3,51]Plant & Microbial Biology, UC Berkeley, [2]Data Science, UC Berkeley, [3]Molecular and Cell Biology, UC Berkeley, [4]Biology, Stanford University, [5]Integrative Biology, UC Berkeley, [6]Institute of Genetics, Biological Reserach Center Szeged, [7]Institute of Biochemistry, Biological Reserach Center Szeged

Parasitoid wasps impose one of the strongest evolutionary pressures on *Drosophila*, and infection prevalence can exceed 50% in natural populations. While parasitoid defenses are well characterized in *D. melanogaster*, little is known about how other species of *Drosophila* defend themselves against parasitoids. We previously discovered the horizontal transfer of two widespread bacterial cytotoxins implicated in parasitoid defense in aphids – *cytolethal distending toxing B* (*cdtB*) and *apoptosis inducing protein of 56 kDa* (*aip56*) – into the nuclear genomes of several insect species, including the *D. ananassae* species subgroup. Of these, *D. ananassae* uniquely encodes a single copy *cdtB* and two *cdtB::aip56* fusion genes. Null mutant analysis in *D. ananassae* indicated each of these genes are necessary to mount an immune response against figitid wasp parasitization. However, how these genes mediate protection, and how *D. ananassae* avoids autotoxicity while deploying these eukaryotoxic proteins, remain unknown. To address this gap, we heterologously expressed bacteria- and insect-derived *cdtB* and *aip56* homologs in yeast and in *D. melanogaster*. In yeast, each of the CdtB::AIP56 fusion proteins arrests growth, while insect-derived CdtB has lost its cytotoxic effect. Strikingly, *D. melanogaster* tolerates constitutive expression of endosymbiont-encoded *cdtB*, and expression of each of the *fusion* genes is consistent with cryptic subfunctionalization among the paralogs. Finally, we tested whether these genes are sufficient for parasitoid resistance in *D. melanogaster*. In summary, we found that genes from microbes can be readily co-opted by *Drosophila* species as macromutations that underlie the evolution of novel innate immune modules.

256T **Identification of *trn* enhancers and their contribution to the evolution of *Drosophila* male genital morphology** Javier Figueras Jimenez[1], Joanna F D Hagen[2], Amber M Ridgway[3], Nicolas Tapon[4], Maria Daniela S Nunes[3], Alistair P McGregor[11]Biosciences, Durham University, [2]Biology, John Hopkins University, [3]Biological and Medical Sciences, Oxford Brookes University, [4]The Francis Crick Institute

Male genital structures have evolved rapidly among animal groups with internal fertilization with respect to others aspect of morphology. In some cases, species that have diverged recently can only be distinguished by these structures. However, we still lack knowledge about the genetic mechanisms responsible for the divergence of genital structures. The claspers (or surstyli) are paired genital appendages used by the male insects to grasp females during copulation. The claspers of *Drosophila mauritiana* are 40% larger and have more bristles that those of *D. simulans* despite these species sharing a common ancestor less that 300 000 years ago. We recently showed that the gene *tartan* (*trn*) contributes to the evolution of clasper morphology between these two species. *trn* encodes a leucine-rich repeat transmembrane protein that is known to be involved in boundaries between cells in other tissues and at different stages of the development. *trn* is differentially expressed (spatially and temporarily) between the developing claspers of *D. simulans* and *D. mauritiana*. This indicates that changes in the regulation of *trn* rather than the coding sequence underlie its contribution to clasper divergence. To investigate the regulation of *trn,* we screened the *trn* locus of *D. melanogaster* to search for enhancers.

We identified three regions active in the male genitalia, one of which, U4, was active in a pattern that overlapped with the endogenous *trn* expression. We identified Caudal (Cad) as a candidate transcription factor to regulate U4. We found that the anal plates are lost upon *cad* RNAi knockdown and that this gene may regulate *trn* expression around anal plates to delimit their specification. We then investigated the activity of the U4 sequence of *D. mauritiana*, and found it has expanded activity in the developing claspers compared to the *D. melanogaster* enhancer. This result suggests that there has been evolution of this enhancer in *D. mauritiana.* We are now investigating how differences in Trn expression change compartment boundaries to cause the evolution of genital organ size

257T **The role of segregation distortion driven evolutionary conflict in hybrid sterility in Drosophila** Jackson RidgesSchool of Biological Sciences, University of Utah

Understanding the genetic and molecular basis of speciation is a fundamental and long-standing problem in evolutionary genetics. The USA and Bogota subspecies of *Drosophila pseudoobscura* are among the youngest diverged hybridizations studied. Hybrid F1 males between Bogota mothers and USA fathers display very weak fertility and produce nearly exclusively female progeny from sex chromosome segregation distortion. Previously, a single gene, *Overdrive (Ovd)*, was shown to be necessary for both phenomena. These dual properties of *Ovd* provide the strongest and most direct evidence for the role of genetic conflict involving segregation distortion in speciation. Yet, *Ovd* alone is not capable of generating either hybrid phenotype: there remain two uncharacterized and essential interactors of *Ovd* that are required for hybrid male sterility and segregation distortion in hybrid F1 males. Here, I genetically map an essential X-linked interactor of *Ovd*. I use a long-term introgression mapping strategy that self-selects for hybrid fertility to map this factor. Using this approach, I mapped this factor to an 8kb duplication containing a rapidly evolving non-coding RNA. Unlike Ovd, which is required for both sterility and distortion, this region is essential for causing hybrid male sterility, but not segregation distortion. This separation of distortion and sterility related phenomena, and a germline expressed non-coding RNA as the probable genetic basis, have strong mechanistic and evolutionary implications for our understanding of how intra-genomic conflicts involving selfish chromosomes can serve as the engine of speciation.

258T **Evolutionary history of *CK2bTes*–a sex-linked ampliconic gene family in the simulans clade** Emiliano Martí[1], Ching-Ho Chang[2], Cécile Courret[1], Amanda Larracuente[11]Biology, University of Rochester, [2]Fred Hutchinson Cancer Research Center

Selfish genetic elements (SGEs) are often sources of intragenomic conflict and antagonistic coevolution between sex chromosomes. These interactions can result in rapid sequence evolution, leading to striking differences between closely related species. A common theme across different taxa is the accumulation of ampliconic gene families on sex chromosomes, sometimes involved in cryptic drive and sterility. Previous studies of high-quality genome assemblies in the simulans clade revealed that a duplication of an autosomal gene, *CK2bTes*, to the sex chromosomes, followed by the amplification of X-linked and Y-linked duplicates into multicopy gene families. These multicopy genes show patterns of testis-specific expression and signatures of positive selection, consistent with a history of genetic conflict. *CK2bTes* shares a common ancestor with *Stellate* (Ste) and *Su(Ste)* loci—multicopy sex-linked genes with a history of meiotic drive in *D. melanogaster*, raising the possibility that this gene family also is caught in an arms race, with recurrent bouts of sequence evolution and gene amplification. Here we combine of genomic and population genetic approaches to further explore the gene structure and evolutionary history of this gene family across the three species. Together, these approaches might help us to better understand the importance of genomic conflict shaping the repetitive landscape in genomes of closely related species.

259T **Temporal analysis of Drosophila genomic variation across decades and centuries illuminates known and novel targets of adaptive evolution** Jeremy D Lange[1], Max Shpak[1], Hamid R Ghanavi[2], Heloise Bastide[1], Justin B Lack[1], John E Pool[1], Marcus C Stensmyr[21]University of Wisconsin - Madison, [2]Lund University

Population genetics seeks to illuminate the forces shaping genetic diversity, often based on a single snapshot of genomic variation. However, utilizing multiple sampling times to study changes in allele frequencies may help clarify the relative roles of neutral and non-neutral forces. Here, I describe two temporal population genomic studies focusing on natural populations of Drosophila melanogaster. In the first study, we examine changes in genomic variation for a northeast US population over a 38 year period, from 1975 to 2013, by comparing a panel of 65 lab-maintained lines against hundreds of wild-collected flies. Here, the most extreme example of positive selection was the Cyp6a17/Cyp6a23 locus, where a deletion associated with insecticide resistance jumped from low to intermediate frequency in less than a decade. We inferred that many other adaptation targets were only detectable at the SNP level, rather than the whole-window level.

We also found evidence for strengthening latitudinal clines: northern-associated alleles at clinal outlier SNPs generally increased in frequency across time. In the second study, we illuminate the evolution of genomic variation across more than 200 years (representing perhaps 3,000 generations of evolution), focused on a population from Sweden. This study was enabled by the successful sequencing of individual genomes from two dozen museum-preserved flies representing two time points (early 1800s and 1933), and the comparison of these genomes against modern data sets. The strongest allele frequency change in the 1800s-focused interval was at CHKov1; this timing may favor the antiviral hypothesis over insecticide resistance in explaining positive selection at this gene. In the 1900s-focused interval, the sharpest shift was at the known DDT resistance gene Cyp6g1. That frequency change was rivaled by a novel selection signal at Ahcy, which is expressed in circadian-regulating neurons and impacts life span. These and other results from our studies showcase the potential of temporal population genomics to advance our understanding of evolution at the genetic level in this model system.

260T **Enrichment of hard sweeps on the X chromosome relative to autosomes in six *Drosophila* species** Mariana Harris[1], Bernard Y Kim[2], Nandita Garud[3,41]Department of Computational Medicine, UCLA, [2]Department of Biology, Stanford University, [3]Department of Ecology and Evolutionary Biology, UCLA, [4]Department of Human Genetics, UCLA

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 the proportion of hard sweeps, in which a single adaptive mutation rises to high frequency, is higher 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 the proportion of soft sweeps, in which multiple haplotypes rise to high frequency simultaneously, is higher on the autosomes. Using haplotype homozygosity statistics, 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. We generalize these findings by analyzing diversity patterns across six *Drosophila* species, where we find consistently steeper reductions in diversity on the X as compared to autosomes. Simulations suggest that these reductions in diversity on the X are most consistent with hard sweeps, indicating that harder sweeps are more common on the X chromosome as compared to autosomes across multiple species of *Drosophila*.

261T **Investigating enhancer and protein divergence at *follistatin* paralogs underlying genetic assimilation of wing plasticity** Kevin D Deem, Jennifer A BrissonBiology, University of Rochester

Over the past several decades, we have gained considerable insight into how developmental gene regulatory networks produce morphological traits, bridging the gap between genotypic and phenotypic diversity between species. What remains unclear is how new traits might arise from environmentally induced variation within a single species. Some have proposed that genetic assimilation, a process by which environmentally induced phenotypes become entirely genetically determined, is a common mode of morphological innovation. However, the underlying mechanisms and evolutionary constraint on genetic assimilation in nature remain virtually unknown. In the pea aphid (*Acyrthosiphon pisum*), evidence suggests that separate duplications of the gene *follistatin* (*fs*) are responsible for female wing polyphenism (*fs-fem*, arose first) and its genetic assimilation in males (*fs-male*, arose second). My work focuses on the functional characterization of *cis*-regulatory and protein-coding sequence at *fs* paralogs, and the evolutionary forces acting on them, to explore the mechanisms of, and constraint on genetic assimilation of wing polyphenism in pea aphids.

The ancestral function of Fs proteins is to inhibit the TGF-beta ligands Myoglianin and Activin-beta (Myo and Act-beta). One possibility is that pea aphid *fs* paralogs have evolved induce winglessness via derived repression of another TGF-beta ligand, Decapentaplegic (Dpp), which is crucial for wing formation. Another possibility is that pea aphid *fs* paralogs rely on ancestral repression of Act-beta and Myo to partially block metamorphosis and growth of wing tissue. To analyze the potential contribution of protein evolution to the roles of *fs-fem* and *fs-male* in wing polyphenism and genetic assimilation, I have overexpressed pea aphid Follistatin (Fs) protein paralogs and assessed their ability to repress Myo, Act-beta, and Dpp in *Drosophila.*

To investigate how enhancer evolution may have modified gene regulation between the two paralogs, I use an integrative approach involving machine learning and available open-chromatin profiling data. I have identified candidate enhancers at *fs-fem* and *fs-male* which exhibit changes in line with our hypothesis, and plan to test the *in vivo* activity of

these enhancers in pea aphids and the fruit fly *Drosophila melanogaster*. This work aims to provide important insights into the molecular mechanistic basis of genetic assimilation, where control of trait variation is shifted from environment ("nurture") to genotype ("nature").

262T **The Evolution of DNA Repeat Sequences at Inversion Breakpoints and TAD Boundaries** Dynisty Wright, Stephen SchaefferThe Pennsylvania State University

Drosophila pseudoobscura is polymorphic for over 30 different paracentric inversions on the third chromosome (Muller C), which have persisted for at least 500,000 years in populations. Breakpoints of six of these inversions were shown to occur at the boundaries of topologically associated domains (TADs) more frequently than expected by chance. These breakpoints were also found to co-occur in regions with small repeats initially discovered in the *D. pseudoobscura* reference genome that carries the Arrowhead inversion suggesting that repeats may play a role in generating new inversions through ectopic exchange as well as playing a role in chromatin structure. If these repeats play a role in chromatin architecture, we expect that their locations in the TAD boundaries would be conserved across the 13 homologous breakpoint regions of the six different inversion types: Tree Line (TL), Cuernavaca (CU), Pikes Peak (PP), Standard (ST), Chiricahua (CH), and Arrowhead (AR). Paired end sequencing and Illumina short read re-sequencing data was able to map inversion breakpoints, but was unable to assemble the breakpoint or some intergenic sequences because of repetitive DNA. Here, we present the analysis of long read assemblies of six *D. pseudoobscura* genomes that differ in the inversion that they carry (AR, ST, PP, CH, CU, and TL). These data were used to test whether short repeat sequences are maintained in breakpoint regions across the six different persisting inversions and to understand how repeats evolve within the breakpoints across arrangements. The genomes were sequenced using Oxford Nanopore and Illumina technologies and were assembled using with the Flye assembler, remapped using long-read data, and polished with Pilon using the AR assembly as a reference. Sequences for the 13 breakpoints were extracted from the Muller C contigs of each inversion type including 1 kb upstream downstream from the intergenic breakpoint region. The 78 breakpoint sequences were collected in a BLAST database and were compared against each other. Our analysis found that the majority of breakpoint sequences have short repeats that match those in non-homologous breakpoints. Repeats are more abundant in the breakpoints of recently derived inversions than those of older derived inversions suggesting that repeats are lost from breakpoints over time and that the repeats play a minimal role in maintaining TAD structure.

263T **Characterizing Genetic Variation in Morphological Scaling** austin wilcox[1], Isabelle Vea[2], Anthony Frankino[3], Alexander Shingleton[21]Biological Sciences, University of Illinois at Chicago, [2]University of Illinois at Chicago, [3]university of Houston

Morphological scaling relationships between the sizes of individual traits and the body captures the characteristic shape of a species, and the evolution of scaling is the primary mechanism of morphological diversification. However, we have almost no knowledge of the genetic architecture of scaling, critical if we are to understand how scaling evolves. Here we begin to explore the genetic architecture of population-level morphological scaling relationships – the scaling relationship fit to multiple genetically-distinct individuals in a population – by describing the distribution of individual scaling relationships – genotype-specific scaling relationships that are unseen or cryptic. These individual scaling relationships harbor the genetic variation that determines relative trait growth within individuals, and theoretical studies suggest that their distribution dictates how the population scaling relationship will respond to selection. Using variation in nutrition to generate size variation within 197 isogenic lineages of Drosophila melanogaster, we reveal extensive variation in the slopes of the wing-body and leg-body scaling relationships among individual genotypes. These data allow us to predict how different selection regimes alter scaling and morphology in Drosophila and is the first step in identifying the genetic targets of such selection.

264T **Altered sexual size dimorphism in *Drosophila melanogaster* via artificial selection** Elizabeth Agolli, Alexander W ShingletonUniversity of Illinois at Chicago

The difference between male and female body size (sexual size dimorphism, SSD), is perhaps the most obvious way in which the sexes differ. Research over the last thirty years has established the developmental, genetic, and physiological mechanisms that regulate body size, much of which has been done in Drosophila melanogaster. Nevertheless, the developmental mechanisms upon which selection acts to generate these observed differences in female and male body size are not well understood. Indeed, the connection between sex determination mechanisms and phenotypic differences in body size remain essentially a black box, representing a fundamental hole in our understanding of SSD. Through artificial selection, I have successfully generated lineages of increased and decreased SSD. I will analyze these

lineages developmentally, physiologically, and genetically to better understand the mechanisms regulating SSD.

265T **Using hybrid swarms to test for co-adaptation of mitochondrial and nuclear genes in *Drosophila*** Leah Darwin[1,2], Faye Lemieux[2], David Rand[21]Center for Computational Molecular Biology, Brown University, [2]Department of Ecology, Evolution and Organismal Biology, Brown University

The mitochondrion is essential to the production of energy within eukaryotic cells across the tree of life. However, mitochondrial genomes (mtDNA) are subject to the accumulation of deleterious mutations because they replicate asexually and without recombination. Mitochondrial and nuclear genomes encode for genes that function together as a part of the oxidative phosphorylation system (OXPHOS) which produces the majority of cellular ATP. Given the system's importance to cellular function and its co-dependence on mitochondrial and nuclear genes, many have hypothesized that epistatic interactions between these two genomes preserve the function of the mitochondrial genome. The mitonuclear co-adaptation hypothesis suggests that response to selection is facilitated by epistasis between nuclear-encoded mitochondrial (N-mt) genes and their respective mitochondrial genome. Prior work has demonstrated the importance of admixture as a component of adaptive evolution, however the importance of admixture in correcting for deleterious mutations accumulated by mtDNA is largely unexplored. In this work, we develop an approach to quantify the role of co-adaptation in the evolution of admixed individuals using genome-wide allele frequencies over 10 generations of outcrossing in very large "hybrid swarm" populations. The outcrossed source populations were initiated from two replicate cages of flies that contain 24 different strains of *D. melanogaster*: 20 wild lines from Zimbabwe or Beijing and 4 additional lines carrying mtDNA from different species (3 from *D. simulans* and 1 from *D. yakuba*). The design of this study provided a replicate source of diverse nuclear and mtDNA variation with deep mtDNA divergence that contributes to fitness differences. To test for the presence of co-adaptation, we used a selective pressure in the form of the insecticide, rotenone, which targets mitochondrial complex I in the OXPHOS system. After multiple additional generations of selection on rotenone, sequence analyses quantified haplotype frequencies of nuclear and mtDNA loci. We use these data to test the hypothesis that the epistasis between nuclear and mtDNA genes encoding complex I of the OXPHOS system underly the response to selection. The data also unveils novel epistatic interactions that warrant future functional analysis and validation.

266T **Effects of D. subobscura Atpα intra-protein background on CG-tolerance adaptation** Flora Borne, Andrew Taverner, Peter AndolfattoColumbia University

One of the main current challenges to understand how species adapt to new environments is to understand the factors that constraint evolution. A large number of studies have focused on understanding the resistance to toxic cardiac glycosides (CG) in animals. It is now well known that resistance evolves multiple times by target site insensitivity of the NA+, K+ - Atpase. In insects, three amino acid changes in the alpha subunit (Atpα) have been repeatedly found to be associated with CG tolerance (at sites 111, 119, 122). Recent works using engineered Drosophila flies have shown that the frequently observed substitutions at sites 111 and 122 have severe neurological defects in the CG-sensitive species D. melanogaster but that the substitution at site 119 which has little effect on CG tolerance can partially rescue the negative pleiotropic effects of sites 111 and 122. Combined, these three substitutions confer CG-tolerance. However, they cannot fully explain the adaptive path leading to CG-tolerant species. Interestingly, the closely related species D. subobscura naturally harbors several combinations of these sites, suggesting that no negative effects are associated with those changes in this species. D. subobscura Atpα protein only differs from 19 amino acids. In order to identify potential additional permissive changes, we engineered the local D. subobscura protein background into D. melanogaster. Importantly, we homogenized the rest of the genome using an inbred line from the DSPR in order to control for genomic background effects. Our results suggest that adding the D. subobscura changes partially rescue the negative effects of the different combinations of sites 111, 119 and 122 but not fully. Although those sites have not been previously reported to have potential effects on target site insensitivity, we also found that those protein background changes have an effect on CG-tolerance. In particular, flies with the D. subobscura background without the three substitutions survived better than flies with the D. melanogaster background on CG supplemented medium. Our results suggest that the adaptive path leading to CG-tolerant species, at least in the Drosophila genus, is relatively complex and involves more than three sites. In particular, it would involve several permissive mutations that are not fully neutral but have a small positive effect on resistance. Our work underlies the important contribution of intra-protein background in constraining species adaptation.

267T **Deleterious consequences of evolutionarily mismatched centromeric histone (Cid) genes in D. melanogaster** Aida De La Cruz[1], Emily Coelho[2], Andrea E Carroll[1], Amy Chen[1], Benjamin Ross[3], Harmit Malik[11]Basic

Sciences, Fred Hutchinson Cancer Research Center, [2]University of Utah, [3]University of Washington

Centromeric histone H3 (*Cid* in Drosophila) is necessary for proper chromosome segregation. Despite its essential role, *Cid* evolves rapidly in Drosophila species. Centromere drive, in which chromosomes compete for transmission in female meiosis via their centromeres, has been proposed as an explanation for this paradoxically rapid evolution of *Cid*. Under this model, *Cid* evolves rapidly to suppress centromere-drive and its deleterious consequences. However, it is unclear what the deleterious consequences that drive rapid *Cid* evolution are. To address this question, we have swapped *Cid* alleles in *D. melanogaster* (*Cid-mel*) with those from *D. simulans*(*Cid-sim*), a closely related species, or from the hypothetical *D. melanogaster- D. simulans* ancestor (*Cid-msa*). We observed lower viability, haploinsufficiency, and increased development time of *D. melanogaster* flies encoding homozygous for either *Cid-sim* or *Cid-msa* relative to *Cid-mel*. Although *Cid-sim* and *Cid-msa* replacement strains were fertile, crosses between *Cid-sim* or *Cid-msa* parents produced few to no viable adult progeny. Cytological studies reveal that this inviability is the result of catastrophic embryonic mitotic defects. To identify which residues are essential for species specific *Cid* function, we have created and are testing chimeras between *Cid-mel* and *Cid-msa* using fertility assays and microscopy. Our studies reveal that rescue of embryonic mitosis is the primary driver behind *Cid* evolution in Drosophila. As centromeric satellites diverge due to centromere-drive, *Cid* proteins must evolve to restore mitotic defects that result because of centromere drive.

268T **Precise inference of natural selection with a genomic dataset spanning Family Drosophilidae** Bernard Y Kim, Dmitri Petrov, Sofia BeskidBiology, Stanford University

In a large collaborative effort with many members of the fly community, we are working to systematically and comprehensively sequence most of the >4,400 species of the model family Drosophilidae. In addition to assembling new genomes, we are generating population genomic datasets by sequencing a mixture of inbred lines and flies collected from the wild. Our goal for the next 16 months is to sequence the genomes of 1,000+ species and to generate population genomic datasets for 200+ species. This will be a powerful genomic resource for the scientific community, enabling genomic analyses at unprecedented resolution through the saturation of both comparative and population genomic data at nearly every possible nucleotide site. Here, we show that this saturation regime allows us to confidently identify signatures of natural selection at the resolution of single genes or even protein domains, to detect systematic shifts in natural selection between drosophilid species groups, and to study natural selection on genes specific to Drosophila tissues or cell types. This work is the first step in building a clade-scale genomic toolkit for dissecting signatures of evolution on individual genes, through the fly body, and across macro-evolutionary time scales.

269F **Evolutionarily novel expression of Marf1 in the D. melanogaster accessory gland is functionally significant** Tiezheng Fan, David BegunDepartment of Evolution and Ecology, University of California, Davis

Novel biological function may be derived from new genes or from changes to the sequence or expression patterns of old genes. For example, ancient genes that acquire novel, derived expression patterns, a process known as gene co-option, may play a role in novel phenotypes. Recent work has identified the gene Marf-1(Meiosis Regulator And mRNA Stability Factor 1) as a potential case of an ancient gene co-opted into male reproductive function in D. melanogaster. Marf-1 is expressed in the male accessory gland in D. melanogaster, yet multiple outgroups show no evidence of expression in this organ, suggesting that the gene became transcriptionally active in the D. melanogaster accessory gland only within the last 2 million years. Does this gene function in D. melanogaster male reproduction, and if so, how has it altered the biology of the accessory gland? Here we report several experiments investigating phenotypes of knockout and knockdown males. We find that these males have reduced fertility, diminished induction of female post-mating responses, and deficits in sperm competitiveness. These results suggest that the novel expression of this gene in the accessory is accompanied by a novel biological function, the details of which remain to be determined.

270F **A combination of developmental mechanisms drives the evolution of *Drosophila* germ granules** Dominique A Doyle[1], Florencia Burian[1], Bianca Ulrich[1], Gissel Hildago[1], Christopher J Sottolano[2], Bianca M Ortega[1], Matthew G Niepielko[1,31]School of Integrative Science and Technology, Kean University, [2]Center for Computational and Integrative Biology, Rutgers University, [3]Department of Biological Sciences, Kean University

The co-packaging of mRNAs into ribonucleoproteins (RNPs) is a conserved strategy for post-transcriptional gene regulation. In many animals, the formation of complex RNPs called germ granules is essential for the regulation of mRNAs that are required for germline development, maintenance, and function. In *Drosophila*, germ granules are assembled at the posterior of the oocyte and function in primordial germ cell development. In *D. melanogaster*, mRNAs accumulate in germ granules by forming homotypic clusters, which are distinct aggregates that contain multiple transcripts of

a specific gene and are nucleated by the master organizer Oskar (Osk). Homotypic clusters in *D. melanogaster* are generated through a stochastic seeding and self-recruitment process that requires the 3' UTR of germ granule mRNAs. We hypothesize that evolutionary changes found in the 3' UTR of germ granule mRNAs impacts their germ granule accumulation. To test our hypothesis, we first investigated the homotypic clustering of two germ granule mRNAs, *nanos* (*nos)* and *polar granule component* (*pgc*) in four *Drosophila* species. By combining single molecule *in situ* hybridization (smFISH), super-resolution confocal microscopy, and quantitative image analysis, we discovered that the mRNA content of *nos* and *pgc* homotypic clusters, as measured by absolute transcript number, were diverse among *Drosophila* species. Specifically, we found that there was up to a 50% difference in the number of transcripts found in *nos* and/or *pgc* homotypic clusters between *D. melanogaster*, *D. pseudoobscura, D. virilis*, and *D. nebulosa*. By integrating biological data with computational modeling, we recapitulated the diversity in germ granule mRNA content from all species tested and discovered that a combination of mechanisms, including changes in *nos*, *pgc*, *osk* levels, and diversity within the *nos* 3' UTR contribute to generating germ granule diversity. Our findings demonstrate how germ granule assembly is subject to evolutionary changes at various and combined mechanistic levels. More broadly, our analysis may provide insight into the processes that govern the diversification of other classes of biomolecular condensates.

271F **Transcriptome analysis in *Drosophila guttifera* reveals candidate genes involved in the specification of a novel color pattern by the Wingless morphogen.** Yuichi Fukutomi[1,2], Airi Sato[2], Rahayu Pertiwi[2], Aya Takahashi[2], Shuji Shigenobu[3], Shigeyuki Koshikawa[41]UC Davis, [2]Tokyo Metropolitan University, [3]National Institute for Basic Biology, [4]Hokkaido University

Diverse color patterns have evolved in various animal taxa. Theoretical models assume that diffusion of signaling molecules such as morphogens can serve as a mechanism for specifying these patterns (Turing 1952). These models assume interactions between an activator and an inhibitor; however, to date there are no systems where both these players have been identified. To understand the evolution of color patterns, we need to identify the genes responsible for both pattern formation and pigment synthesis. *Drosophila guttifera* has a novel polka-dotted pattern of wing melanin pigmentation, induced by localized *wingless* (*wg*) morphogen expression. Overexpression of *wg* in *D*. *guttifera* pupal wings produces ectopic pigmentation. Other species, such as *D*. *melanogaster*, have unpigmented wings despite expressing *wg,* suggesting that the origin of wing pigmentation in *D. guttifera* involved incorporation of melanin synthesis genes into the gene network downstream of *wg*. Indeed, expression of one of these genes, *yellow* (*y*), is observed in presumptive wing spots and is activated by *wg*. However, overexpression of *y* in *D. melanogaster* wings does not produce pigmentation, indicating that additional melanin synthesis genes must be regulated downstream of *wg* in *D. guttifera*. Identifying these genes is essential for understanding the developmental mechanism responsible for the emergence of the novel color pattern. To accomplish this, we performed transcriptome analysis in *D. guttifera* pupal wings. We compared presumptive pigment spots and regions where *wg* was expressed ectopically to unpigmented wing regions that lacked *wg* expression. We found that several melanin synthesis genes including *y, tan*, and *laccase2* were upregulated in future wing spots and positively regulated by *wg*. Other pigmentation genes such as *silver*, *yellow-e*, and *yellow-h* were downregulated in future spots and negatively regulated by *wg*. We also found that *DWnt4*, a diffusible antagonist of *wg,* is expressed in presumptive wing spots and is upregulated by *wg*. As *DWnt4* is expressed near the *wg* expression site in *D. melanogaster* wings, the regulatory relationship between *wg* and *DWnt4* may be conserved in unspotted species. We hypothesize that *DWnt4* may inhibit wing pigmentation in *D. guttifera*, and that *wg* and *DWnt4* may correspond to the activator and inhibitor of the Turing model. We will use CRISPR/Cas9-induced somatic mutations to test this hypothesis.

272F **A new *Drosophila* genome database for evolutionary research** Bing-Ru Lee[1], Sheng-Yu Chang[2], Chau-Ti Ting[3], Wei-Sheng Wu[11]National Cheng Kung University, [2]National Taiwan University, [3]Life Science, National Taiwan Univ

Drosophila melanogaster, a classic model organism, has been studied for more than one hundred years. Many important genetic and molecular processes were understood by studying the function of a specific gene or a group of the genes in *D. melanogaster*. By comparing the orthologous genes between related species, we can reveal the evolutionary history of genes and identify important innovations can be revealed. However, even with the accessibility of the orthologous gene prediction database and genome browser, it is still quite challenging to compare the gene annotation across these species. Here, we present a *Drosophila* database displaying gene synteny of 18 fly species. We identified the orthologous genes by comparing the similarity between protein and gene sequence. Using D3.js, the JavaScript toolkit, we also provide the gene information and result of ortholog prediction. The database offers a gene annotation table for each species with good-quality assembled genomes. Scientists can search the target gene by gene name, position, protein function, and other gene features from the table. The target gene and its ortholog will be presented in the function of

"gene order," and users can easily compare the gene synteny across multiple genomes simultaneously.

273F **Coevolution between two essential telomere-binding proteins preserves female fertility** Hannah Futeran[1], Sung-Ya Lin[1], Mia Levine[1,21]Biology, University of Pennsylvania, [2]Epigenetics Institute, University of Pennsylvania

Telomeres consist of repetitive DNA sequence and specialized telomere-binding proteins. These nucleoprotein structures ensure that the unique genetic information at the chromosome ends is replicated completely and that chromosomes do not fuse end-to-end. Impairing either function results in cell death. Given the important roles of telomeres, the proteins responsible for mediating these functions are expected to be conserved across species. However, some of these proteins are instead evolving rapidly under positive selection. This is the case for nearly half of the proteins required for telomere function in *Drosophila*, making the *Drosophila* telomere an excellent model for exploring the paradox of essential, conserved biological functions supported by unconserved proteins. Among these adaptively evolving proteins are telomere-binding proteins HipHop and HOAP, which protect telomere ends from fusions. These proteins physically interact and are interdependent for protein stability, raising the possibility that HipHop and HOAP coevolve to preserve telomere integrity. To test this possibility, we used CRISPR/Cas9 to replace the native *hiphop* allele in *D. melanogaster* with a highly diverged version from a close relative, *D. yakuba (hiphop[yak])*. We found that *hiphop[yak]* flies are homozygous lethal, but viability is restored upon the reintroduction of the *D. yakuba* version of its binding partner, HOAP (HOAP[yak]). Intriguingly, we also observed fitness defects in *hiphop[yak]* heterozygotes: female fertility, but not male fertility, is significantly reduced. *HOAP[yak]* also rescues *hiphop[yak]* female heterozygote fertility. Moreover, overexpressing *hiphop[yak]* in the female germline leads to complete sterility, while overexpressing *hiphop[yak]* ubiquitously has no viability cost. These results suggest that *hiphop[yak]* acts as a dominant negative allele in the female germline and/or early embryo but not in the soma. To probe the mechanistic basis of impaired female fertility, we quantified fecundity and DNA damage in the ovaries of the heterozygotes. While there was no significant difference in fecundity, preliminary data suggest that *hiphop[yak]* has elevated rates of double-strand breaks. To determine if this DNA damage impacts a later developmental stage, we are now focusing on the early embryos of these mothers. The results will reveal the precise development stage and possible mechanisms by which telomere protein-protein coevolution preserves genome integrity.

274F **Metabolism as an engine of functional environmental and evolutionary responses across levels of biological organization** Kristi Montooth[1], Ibrahim El-Shesheny[2,3], Omera Matoo[2,4], John DeLong[21]School of Biological Sciences, University of Nebraska-Lincoln, [2]University of Nebraska-Lincoln, [3]Tanta University, [4]University of South Dakota

Temperature has a profound effect to accelerate biological rates from the biochemical to the developmental. Yet, it is less well understood what underlies the failure of cellular, organismal, and population-level processes at higher temperatures, many of which have critical thermal limits that are much lower than the temperatures at which proteins denature. This is critical to understand because many ectothermic species have maximal population growth rates at temperatures that are close to the critical thermal limit, resulting in small safety margins as temperatures warm. We used an outbred population of *Drosophila melanogaster* maintained at 22C to fit thermal performance curves (TPCs) for mitochondrial physiology, metabolic rate, and life-history traits that we then used to construct life tables and estimate the TPC for population growth rate. We found that the thermal optimum for mitochondrial respiration coincided with the thermal optimum for fecundity around 22C. While traits like metabolic rate and development rate continued to accelerate with temperatures beyond this thermal optimum, mitochondrial leak increased, and survivorship and fecundity declined. The TPC for population growth rate emerged not only from the thermal sensitivity of the underlying life-history traits, but from the temperature-dependent integration of these traits. For example, we found that the relationship between parental age and offspring survivorship was itself temperature dependent – flies developed at 18C maintained higher offspring survivorship across a greater fraction of their lifespan. Finally, the thermal sensitivity of the rising portion (activation energy E_a) of TPCs increased, TPCs got narrower, and performance decline at higher temperatures became steeper across increasing levels of biological organization. We then evolved this population in replicate to warmer and cooler temperatures to investigate whether metabolism and mitochondrial physiology changed in ways that facilitated shifts in TPCs at higher biological levels of organization during thermal adaptation. In summary, we find that metabolism and development speed up dramatically with temperature, but at a cost to individual performance traits that shape population growth rate. The thermal sensitivity of the mitochondria appears to be a critical component underlying costs associated with accelerating the pace of life in ectotherms; and, in fact, may be the Achilles' heel that limits ectothermic thermal tolerance and adaptation as suggested by Dahlhoff & Somero (1993) and Somero et al. (2016).

275F **New gene evolution with subcellular expression patterns detected in PacBio-sequenced genomes of Drosophila genus** Chuan Dong[1], Li Zhang[1], Shengqian Xia[1], Dylan Sosa[1], Deanna Arsala[1], Andrew G. Clark[2], Rod A. Wing[3], Manyuan Long[11]The University of Chicago, [2]Cornell University, [3]The University of Arizona

Previous studies described gene age distributions in the focal species of Drosophila melanogaster. Using third-generation PacBio technology to sequence Drosophila species we invesitigated gene age distribution in the two subgenera of Drosophila. Our work resulted in four discoveries. First, our data detected abundant new genes in entire Drosophila genus. Second, in analysis of subcellular expression, we found that new genes tend to secret into extracellular matrix and are involved in regulation, environmental adaption, and reproductive functions. We also found that extracellular localization for new genes provides a possible environment to promote their fast evolution. Third, old genes tend to be enriched in mitochondria and the plasma membrane compared with young genes which may support the endosymbiotic theory that mitochondria originate from bacteria that once lived in primitive eukaryotic cells. Fourth, as gene age becomes older the subcellular compartments in which their products reside broadens suggesting that the evolution of new genes in subcellular location drives functional evolution and diversity in Drosophila species. Finally, we showed that a gene traffic out of the X chromosome also occur in the Drosophila subgenus, suggesting this is a genus-wide phenomenon in Drosophila.

276F **Characterizing putatively adaptive *P*-element insertions in recently invaded laboratory *D. melanogaster* populations** Savana HadjipanteliBiology and Biochemistry, University of Houston

In the 1950s, *P*-element DNA transposons invaded the Drosophila melanogaster genome. Especially upon initial invasion, transposable elements (TEs) have a predominantly negative effect on host fitness, due to both DNA damage arising from transposition and associated fertility effects. However, TEs also hold the potential to generate novel beneficial alleles. In an effort to recreate and study the initial host response to novel TEs, we introduced *P*-elements into a naive laboratory population via germline transformation. We observed a rapid expansion of genomic *P*-elements, followed by the evolution of piRNA mediated repression, which parallels the outcome of *P*-element invasion in natural populations. However, we also observed the recurrent adaptive insertion of *P*-elements into a largely uncharacterized non-coding RNA, *lncRNA:CR43651*. The fitness advantage conferred by these insertions is puzzling, as *lncRNA:CR43651* is not a known genomic source of piRNA, and high-frequency putatively adaptive insertions are not observed at this locus in natural populations.

We are currently testing two alternative hypotheses to explain the fitness benefit of *lncRNA:CR43651* insertions in laboratory populations. Our first hypothesis is that these insertions are repressing *P*-element activity through piRNA silencing within germline stem cells (GSCs). *P*-element transposition occurs predominantly in pre-meiotic germ cells, and it has recently been shown that these cells may rely on distinct piRNA clusters that have gone undetected in whole ovary samples. Using a lacZ reporter gene, we are visualizing *P*-element activity in GSCs in the presence of *lncRNA:CR43651* insertions to address the potential for piRNA repression. Alternatively, *lncRNA:CR43651* insertions may provide fitness benefits unrelated to *P*-element regulation by impacting the expression of *mir-14* transcripts derived from *lncRNA:43651* precursors. Changes in *mir-14* expression may have beneficial effects on life-history traits under laboratory conditions, as *mir-14* has functions in stress tolerance, cell-death regulation, hormone production, fat metabolism, and aging. To address this alternative, we are characterizing changes in development-time and fecundity associated with *lncRNA:43651* insertions. Our experiments will reveal the beneficial effects of *lncRNA:43651* insertions, as well as providing an example of how an invading TE can fuel adaptation through producing novel beneficial mutations.

277F **Repression precedes independent evolutionary gains of a highly specific gene expression pattern** Jian Pu, Zinan Wang, Henry ChungMichigan State University

Highly specific expression patterns can be caused by the overlapping activities of activator and repressor sequences in enhancers. However, few studies illuminate how these sequences evolve in the origin of new enhancers. Here, we show that expression of the *bond* gene in the semicircular wall epithelium (*swe*) of the *Drosophila melanogaster* male ejaculatory bulb (EB) is controlled by an enhancer consisting of an activator region that requires *Abdominal-B* driving expression in the entire EB and a repressor region that restricts this expression to the EB *swe*. Although this expression pattern is independently gained in the distantly related *Scaptodrosophila lebanonensis* and does not require *Abdominal-B*, we show that functionally similar repressor sequences are present in *Scaptodrosophila* and also in species that do not express *bond* in the EB. We suggest that during enhancer evolution, repressor sequences can precede the

evolution of activator sequences and may lead to similar but independently evolved expression patterns.

278F ***Chromosomal Inversion enables* Drosophila's *seasonal adaptation by modulating behavior*** Benedict A Lenhart[1], Alan Bergland[2], Joaquin Nunez[2], Connor Murray[2], Alyssa Bangerter[21]Biology, University of Virginia, [2]University of Virginia

Drosophila melanogaster populations adaptively track to seasonal fluctuations in selection pressure, leading to genetically based phenotypic shifts in in multiple quantitative traits. However, we lack an understanding of the functional genetic architecture of this rapid evolutionary change. Inversions are a plausible genetic mechanism of rapid evolutionary change of multiple phenotypes because they can accumulate linked variation at multiple genes and are therefore likely to be pleiotropic. We tested this hypothesis by studying the phenotypic effects of a cosmopolitan inversion, In(2L)t. This inversion has been hypothesized to underly rapid seasonal change, and its frequency is strongly correlated with temperature in the weeks prior to sampling. Using a meta-analysis of over 200 phenotypes collected on the DGRP, we show that In(2L)t has a significant effect on dozens of phenotypes, and is especially enriched for behavioral phenotypes. In(2L)t status explains a multivariate phenotype defined by the first principal component of the traits associated with the inversion, with inverted lines showing greater activity and lifespan, and non-inverted lines showing larger body size and frequency of sleep. To identify the candidate regions linked to the inversion that underly seasonal adaptation and phenotypic variation, we combined the signal of temperature dependent allele frequency change with signal of association between genotype and phenotype for these traits. We identified several regions, including the In(2L)t breakpoint, as strongly enriched for SNPs that are both seasonal and associated with phenotype. One of the phenotypes that is most strongly affected by In(2L)t status is startle response. Startle response, and activity more generally, is also believed to play a role in survival to harsh conditions. To test the effect of candidate loci on startle response and to further resolve the genetic basis of rapid evolutionary change, we performed a deficiency mapping assay targeting the inversion breakpoint plus 2 other candidate loci inside the inversion. We find that genetic variation at a small genomic region inside the inversion and physically close to the distal breakpoint is associated with startle response. This same region is highly pleiotropic, suggesting that it might contribute to rapid evolutionary change in a suite of highly correlated traits.

279F **One-third of *Drosophila* orphan genes are putative *de novo* genes** Shengqian Xia[1], Zihan Liang[2], Yuxin Peng[3], Dylan Sosa[1], Chunyan Chen[4], Yong E. Zhang[4], Wei Zhang[5], Jian Zu[3], Li Zhang[1], Shengqian Xia[61]The University of Chicago, [2]Chinese Institute for Brain Research, [3]Xi'an Jiaotong University, [4]CAS, [5]Peking University, [6]University of Chicago

De novo genes originating from non-coding sequences are an important, understudied category of genes whose study illuminates the processes underlying how genetic novelties arise from random sequences. Prior work has shown that *de novo* genes have male biased expression and play important roles in male fertility. However, the validity of genes annotated as *de novo* remains controversial as evidence of stepwise *de novo* origination from non-coding sequence to coding is usually absent. In most cases, orphan genes consist of fast-evolving old genes and *de novo* genes, are incorrectly annotated as *de novo* genes. In this study we identified 41 *de novo* genes in the *D. melanogaster* genome emerging within 50 million years. These *de novo* genes are significantly deficit on the X chromosome and show an early-ORF-late-transcription pattern. Moreover, they are less optimized for both transcription and translation. With machine learning classifiers built on them we predicted 31 putative *de novo* genes out of 89 orphan genes. Five recently derived putative *de novo* genes were selected for functional analyses. They were detected to be involved in wing development, male fertility, and lifespan; although the effects were not always beneficial. Overall, our results provide a reliable dataset of *Drosophila de novo* genes. Based on this, we find that *de novo* genes make up one-third orphan genes and contribute to phenotypic diversity in *D. melanogaster*, which provides new insights into the functionality of *de novo* genes.

280F **Modeling satellite DNA organization** Sherif Negm[1,2], Emilliano Martí[2], Amanda M Larracuente[21]Human Genetics, The University of Chicago, [2]Biology, University of Rochester

Repetitive DNAs comprise large portions of eukaryotic genomes. Satellite DNAs (satDNAs) are abundant tandemly repeated DNA sequences found near centromeres, telomeres, and on sex chromosomes. SatDNAs originate through polymerase slippage, recombination between repeat elements, or TE-mediated mechanisms. Arrays of satDNA repeats are highly dynamic over short periods of evolutionary time: they vary in copy number and organization through unequal exchange, and other processes. SatDNA array expansion is thought to decrease organismal fitness but the relative importance of processes shaping satDNA evolution in natural populations is poorly understood. Population genetics studies have primarily focused on estimated copy number variation in satDNA arrays, due in part to limits in empirical

data, as the repetitive nature of satDNAs make them difficult to study in detail. Recent advances in DNA sequencing now make it possible to infer satDNA organization at the sequence level, providing a richer source of empirical information. Here we provide a novel population genetics approach to study sequence variation in satDNA arrays. We simulate the effects of mutation, unequal exchange, gene conversion, drift, and selection on satDNA array sequence, structure, and organization in populations in a forward simulation framework. We designed a new probabilistic model for unequal exchange and gene conversion that takes into account sequence divergence between monomers in the repeat array. We have identified summary statistics that can be used to estimate unequal exchange rate and gene conversion shaping the evolution of tandem repeats. We have applied our model to obtain empirical estimates of gene conversion and unequal exchange from natural populations.

281F **Motor circuit evolution: Muscle pattern diversity in Cyclorrhaphan larvae** Annika Sharma[1], Yiqin Gao[2], Elizabeth S Heckscher[31]Neurobiology, The University of Chicago, [2]The College, The University of Chicago, [3]Molecular Genetics & Cell Biology, The University of Chicago

Despite vast diversity in motor behaviors across animals, motor circuits are highly conserved in their general structure and consist of pre-motor neurons, motor neurons, and muscles. Relatively little is known about the evolutionary constraints on motor circuits, partly because we have lacked the means to study all circuit components at single-cell resolution in a closely related group of animals. Here, we propose that the evolution of motor circuits follows a 'bowtie' model wherein motor neuron numbers change more slowly than those of their inputs and outputs. Many possible combinations of changes to the motor circuit can generate a novel pattern of movement; this model contends that changes are likely decoupled, occurring independently in pre-motor networks and muscles. Testing our hypothesis requires us to characterize each motor circuit component at cellular resolution in several closely related species. This poster focuses on our first level of characterization, differences in body wall musculature. We have selected larval Drosophila melanogaster as a useful reference species because its motor circuit components are well-understood at the cellular level. Our data and published work have found no variation in body wall muscle patterns between many species of Drosophilidae, the family that contains D. melanogaster. Thus, Drosophilidae larvae are too similar for our study, which has led us to explore the suborder Cyclorrhapha (insects with soft, segmented, and limbless larval body forms). In Cyclorrhaphan larvae, we see the same three major muscle groups as in D. melanogaster (dorsal, ventral, and transverse/lateral muscles), with variations within each group. We systematically identify anatomically homologous, altered, and species-specific muscles and characterize these differences between taxa by introducing a novel flexible naming scheme. In general, data from our lab suggests that there are two functional types of muscles—those driving peristalsis (a wave-like movement that travels along the body axis and is used for locomotion) and those used for substrate interaction. Our initial results indicate that muscles for peristalsis are more conserved than those involved in substrate interaction. In addition to musculature, we are currently studying motor neuron number and motor neuron-to-muscle connectivity, and pre-motor networks. We expect to identify points of flexibility and constraint in motor circuits over evolutionary time scales.

282F **Analysis of high-resolution recombination rate variation between *Drosophila santomea* and *D. yakuba* based on whole-genome sequencing of individual meiotic events** Nikale Pettie[1], Ana Llopart[2,3], Josep Comeron[1,31]Department of Biology, University of Iowa, [2]Depatment of Biology, University of Iowa, [3]Interdisciplinary Graduate Program in Genetics, University of Iowa

The number and distribution of crossovers across genomes are highly regulated during meiosis, yet the key components controlling them are fast evolving, limiting our understanding of the mechanistic causes and evolutionary consequences of changes in crossover rates. Whereas a high-resolution crossover map for *Drosophila melanogaster* has been available for a decade, crossover data in this well studied model organism lacks evolutionary context. We recently applied a highly efficient approach to generate whole-genome high-resolution crossover maps in *D. yakuba* based on the analysis of individual meiotic events. Here we apply the same approach to generate whole-genome high-resolution crossover maps in *D*. *santomea* to tackle multiple questions that benefit from being addressed collectively within an appropriate phylogenetic framework, in our case the sister species *D. santomea* and *D. yakuba* and the more distantly related *D*. *melanogaster*. We find that the overall genetic map of *D. santomea* (324 cM) is intermediate in length, being longer than *D. melanogaster* (287 cM) and shorter than *D. yakuba* (339 cM). The difference between *D. yakuba* and *D. santomea* is particularly acute in the *X* chromosome, where *D. yakuba* shows a 1.5X greater crossover rate than *D. santomea*. Similar to other *Drosophila* species, we identify a centromere effect in *D. santomea* that is, however, significantly weaker than that observed in *D. yakuba* and more equivalent to that observed in *D. melanogaster*. Estimates of the number of crossovers per tetrad also show patterns that more closely resemble *D. melanogaster* than *D. yakuba*. The large

difference in crossover patterns and rates between two sister species that can produce fertile hybrid females provides a unique opportunity for future studies to dissect the genetic and genomic basis of crossover control.

283F **Let's talk about bruno: Using QTL mapping and CRISPR HDR to uncover causative variants of dysgenic sterility in *Drosophila melanogaster*.** Lorissa Saiz, Erin S KelleherBiology and Biochemistry, University of Houston

Transposable elements (TEs) are widespread genetic parasites that proliferate and increase in copy number in the germlines of organisms to guarantee transmission to offspring. While TEs are predominantly vertically transmitted, they occasionally invade new host genomes via horizontal transfer between species. Invading TEs can greatly reduce the fertility of their new host, creating strong selection on host alleles that rescue fertility. *P*-elements are DNA transposons that were horizontally transferred from *Drosophila willistoni* to *Drosophila melanogaster* in the 1950s. In the absence of piRNA-mediated regulation, *P*-elements cause germline DNA damage, leading to a sterility syndrome called hybrid dysgenesis. Through QTL mapping, we previously identified the *bruno* locus as a likely source of natural variation in female dysgenic sterility. *bruno* is an RNA-binding protein and translational repressor with a significant role in *Drosophila* oogenesis and regulation of germ cell differentiation, making it a strong candidate gene to influence dysgenic sterility. Furthermore, fertile QTL alleles exhibit reduced ovarian *bruno* expression, and *bruno* loss of function alleles are dominant suppressors of dysgenic sterility. However, the original QTL window is too large to determine the causative variant(s), and the sequence differences responsible for the variation in dysgenic sterility between sterile and fertile alleles are unknown. To narrow the QTL window, I am performing fine-scale, marker-assisted mapping of the *bruno* QTL. In the future, I will determine which sequence differences between fertile and sterile alleles influence dysgenic sterility by performing CRISPR-Cas9-mediated allele swaps. Identifying these causative variants will allow us to better understand how differences in ovarian *bruno* gene expression arise and, ultimately, if the reduced expression was beneficial following *P*-element invasion. In this way, *bruno* represents a case study for future understanding of how host modifiers of dysgenic sterility impact the fitness landscape during TE invasions.

284F **Testing the functional relevance of a key herbivore detoxification gene: an "in-fly" approach** Paula J Fernandez Begne[1], Timothy J O'Connor[1], Marcus J Kronforst[21]Ecology and Evolution, University of Chicago, [2]University of Chicago

The evolutionary success of herbivorous insects is thought to be due to the co-evolutionary dynamics with their host plants. In order to feed on chemically defended plants, herbivores must evolve mechanisms to detoxify plant secondary metabolites. In this study, we develop a heterologous "in-fly" system to study the functional relevance and evolution of *nitrile specifier protein* (*NSP*), a protein that Pierinae butterflies use to detoxify the chemical defenses of their host plants Brassicales. Brassicales use the glucosinolate-myrosinase defense system (mustard bomb) to kill herbivores. In most insects, glucosinolates are modified into toxic isothiocyanates by plant myrosinase enzymes after consuming plant tissue. Macroevolutionary patterns and in-vitro experiments suggest that the ability of Pierinae butterflies to feed on Brassicales is due to the *nitrile specifier protein* (*NSP*) gene family. However, the functional importance of *NSP* for protection against glucosinolate defenses has not been directly assessed in-vivo. To evaluate the protective effects of *NSP* against glucosinolates, we used the genetic toolkit of *Drosophila melanogaster* to express *NSP* in an easily genetically manipulable system. *D. melanogaster* is sensitive to isothiocyanates, the toxic byproduct of glucosinolates, and lacks *NSP* genes. In Pierinae butterflies, the re-direction of glucosinolate breakdown products happens in the gut, after disruption of plant cells through chewing. Because the two components need to be separate before consumption, it is currently not possible to rear flies on artificial media with known quantities of glucosinolates and myrosinase. To get around this problem, we constructed transgenic flies that express plant myrosinase enzyme (*Arabidopsis thaliana TGG1*) and *NSP*. A fly that expresses myrosinase ensures that the mustard bomb is not triggered until it ingests glucosinolates. Flies expressing *TGG1* showed low egg-to-adult survivorship when fed with media with allyl glucosinolate, but suffered no detrimental effects on regular media. This result allows us to use our "in-fly" system to test whether expressing *NSP* is sufficient for organismal protection against glucosinolate compounds. Furthermore, if *NSP* is the key mechanism for chemical defense, we will be able to use this system to study *NSP* functional evolution.

285F **TRPN channel diversification: a mechanosensory driver of insect hyperdiversity?** Maurice J Kernan[1], Shao-Kuei Huang[1,2], Karli Casler[1,31]Neurobiology & Behavior, Stony Brook University, [2]Department of Biochemistry and Pharmacology, NYU School of Medicine, [3]Biology, University of Rochester

TRPNs are mechanically-activated ion channels that transduce the physical stimuli giving rise to touch, hearing, vibrosensation and proprioception. First identified in *Drosophila* by *no mechanoreceptor potential C* (*nompC*) mutations,

they are present in mechanosensory cells in most animals. In flies, the NompC/TRPN channel is located in the ciliated neurons of external mechanosensilla and chordotonal organs, and in some multidendritic neurons.

The NompC channel pore is encoded by two paralogous exon sets, which we find to be alternatively spliced as mutually exclusive cassettes, resulting in two channel variants that generate slow- or fast-adapting mechanoreceptor currents when expressed in bristle neurons. *In vivo* splice reporters reveal a highly patterned choice of the alternative exons in individual neurons, which matches their sensory mode and response. Among campaniform sensilla on the wing blade, phasic sensilla, which respond to fast changes in stimulus intensity, exclusively express the fast-adapting isoform; tonic sensilla, which fire throughout a constant stimulus, express the slow-adapting form. Similarly in chordotonal organs of the legs and antennae, the fast-adapting isoform is expressed in groups of neurons that sense high-frequency vibration; the slow-adapting isoform in neurons that report slower, proprioceptive signals.

Multiple sequence comparisons show that these paralogous pore exons originated in a common ancestor of the Diptera, and Lepidoptera. Alternative TRPN pore exons or exon sets also arose independently in several other insect orders, including the hyperdiverse Hymenoptera (sawflies, wasps, ants) and Coleoptera (beetles). Four separate beetle families show further multiplications of the pore cassettes and/or the whole gene, suggesting an unsuspected complexity of their mechanosensory worlds. Among all insects, we infer at least five early and independent origins of TRPN pore variants, with no apparent losses from any descendant clade. This distinctive pattern of mechanosensory evolution contrasts with that of the chemical and visual senses: instead of multiplying receptor genes, mechanosensory complexity in insects arose through repeated variations in the structure of a single channel gene, with variant expression regulated at the level of splicing.

As in *Drosophila* courtship, many insects use species-specific vibroacoustic signals to identify conspecific mates; species in some cryptic groups are distinguishable only by these signals. We suggest that, by enhancing mechanosensory signal discrimination, early TRPN channel diversification could have triggered rapid speciation in multiple insect orders.

286F **Investigating the maintenance of the *Responder* satellite in *Drosophila melanogaster*** Matthew P Lindsay[1], Danna Eickbush[1], Xiaolu Wei[2], Amanda Larracuente[11]Biology, University of Rochester, [2]Biomedical Genetics and Genomics, University of Rochester

Large blocks of repetitive, non-coding satellite DNA (satDNA) are a major component of eukaryotic genomes, but their exact functions or contributions remain unknown. While satDNA is typically seen as 'junk' DNA, some have roles in chromosome segregation and nuclear organization, though few specific functions or fitness effects have been determined. SatDNA may also persist and expand through selfish processes like female meiotic drive that increase the copy number of certain satDNA sequences. To study why satDNA is maintained, we used CRISPR to modify the Responder (Rsp) satellite in Drosophila melanogaster to investigate its specific fitness contributions and potential involvement in female drive. Rsp is the target of the Segregation Distorter (SD) meiotic driver: chromosomes with large Rsp loci (many copies of the Rsp element) are susceptible to destruction by SD, and heterozygous SD males only produce sperm bearing Rsp-lacking SD chromosomes. While complete Rsp deletions are viable and fertile, large, drive-susceptible Rsp loci are common in wild populations. The high frequency of large Rsp loci may be due to a transmission bias in females that causes large Rsp loci to be inherited more frequently than small loci, or by fitness costs associated with small loci. In a previous study, a large deletion including Rsp significantly reduced fitness, suggesting Rsp may serve an unknown but important function. However, this large deletion removes many sequences, making it difficult to attribute fitness effects to Rsp specifically. We used CRISPR to make precise modifications of the Rsp locus, and used PacBio HiFi sequencing to investigate the modified loci. We show that Rsp deletions significantly reduce sensitivity to SD in previously sensitive backgrounds, indicating that we successfully altered the phenotype associated with the Rsp satellite. We are measuring the transmission rates of deletion and ancestral chromosomes in females to determine if large, ancestral loci are transmitted to offspring more frequently than deletion loci, and using cage experiments to measure the fitness of deletion chromosomes against the ancestral chromosomes over many generations in large panmictic populations. These experiments will help explain why drive-sensitive alleles of Rsp persist in wild populations- leaving them vulnerable to SD- and could more broadly help explain why satDNA makes up such a large proportion of eukaryotic genomes.

287F **Variable phenotypes and complex genetic architecture underlie hybrid male sterility between recently-diverged populations of Drosophila melanogaster** Myron B Child[1], Matthew Lollar[1], Eleyna Escobedo[1], Clarice Danen[2], Timothy Biewer-Heisler[2], John Pool[11]Genetics, University of Wisconsin, Madison, [2]University of Wisconsin, Madison

Deciphering how diverging populations become reproductively isolated is crucial to understanding how new species are formed. The majority of speciation studies, however, focus on distinct, relatively well-isolated species, which may miss the early stages of this process and are unable to explore where the first sources of isolation originate. *Drosophila melanogaster* has experienced a recent worldwide expansion, generating populations with varying degrees of pairwise genetic distances and locally adaptive changes. Using inbred strains of *D. melanogaster* generated from recently isolated European and African populations (~13 kya), we find evidence of hybrid breakdown, wherein F2 males generated from between-population crosses show elevated levels of sterility compared to within-population cohorts. Increased sterility rates among crosses are both strain- and cross-direction-dependent, suggesting a variable and sex-specific genetic architecture. This variability is recapitulated at the phenotypic level, with flies displaying variable combinations of testes morphological defects, spermatogenic defects, and ineffective sperm transfer. Mating assays reveal that some hybrids are also deficient in courtship and mate less frequently compared to controls. Preliminary efforts to map pairwise incompatibilities between these populations implicate uniparentally inherited genetic elements from both Africa and Europe, however a lack of sterility in backcross experiments implies that incompatibilities are multi-locus. Overall, our results suggest a variable and complex multigenic basis for reproductive incompatibility between recently-diverged populations, providing a rare window into the earliest stages of isolation.

288F **Germline RNAi of *D. melanogaster double fission* (CG34200) Causes Nurse Cell Doubling** William D. Gilliland, Marcin Marciniak, Olivia Bowen, Kelly Conger, Doreen Elrad, Gabrielle Presbitero, Denny P MayBiological Sciences, DePaul University

We found that germline-specific RNAi knockdown of a previously uncharacterized *Drosophila melanogaster* gene (CG34200) produced a high incidence of oocytes with 31 nurse cells and ring canals instead of the usual 15, presumably due to an extra round of mitosis during early oogenesis. Because of this phenotype, we have named this gene *double fission (dubf).* This gene is only 52 amino acids long with no conserved protein domains, but this sequence is highly conserved among *Drosophila* species, and clear homologs can be found as far back as daphnia. Additionally, BLAST searches identified a closely related uncharacterized gene in *D. melanogaster* (CG15461), which we have named *double fission-related (dubfr).* Phylogenetic analysis shows that *dubfr* was created in a duplication event that occurred around 65 million years ago, prior to the diversification of the Drosophila clade. As no alleles were available of *dubf* except for a single RNAi knockdown construct, we are attempting to generate null alleles by imprecise P-element excision as well as by inducing CRISPR-mediated point mutations. While the P element was originally viable over a deficiency, ~25% of excisions cause lethality over that deficiency. Preliminary evidence is consistent with *dubf* evolving slowly due to it being an essential gene required for viability.

289F **Following Muller's Footsteps: Mapping Natural Variation in Mutagen Sensitivity.** Llewellyn Green, Lillian Pennington, Erin KelleherBiology and Biochemistry, The University of Houston

Organisms are constantly under attack by numerous mutagens in the environment. Apart from their obvious direct effects on DNA damage and mutation, many of these mutagenic agents can ***exert*** other harmful physiological effects on the organism, up to and including death. Therefore, sufficient mutagen tolerance is fundamental to organismal fitness. While many of these agents—such as UV radiation—are close to ubiquitous in the environment, the degree of exposure can vary over space and time. So while it is expected that most organisms would display some degree of adaptation, it is predicted that selection for mutagen tolerance would vary, resulting in different outcomes for genetic diversity. However, there is still much to be learnt about the genetic factors that contribute to natural variation in mutagen tolerance.

To uncover the genetic determinants of mutagen tolerance, we implemented an extreme QTL, pooled-sequencing approach, utilizing a mass-bred population derived from the Drosophila Synthetic Population Resource. We predict that this extreme-mapping approach will give us enough statistical power to map regions associated with mutagen tolerance to a high resolution. For this study, we focus on two common environmental mutagens: ionizing (X-ray) and UVB radiation. These two agents represent an interesting contrast, as while they are both found widespread in the environment, they differ both in their degree of variance and in their intensity. We predict that of the two agents, there will be more heritable variation in UVB tolerance—as terrestrial UVB doses are more intense than background ionizing radiation and generally more geographically variable. This work will have significant implications for how organisms withstand the onslaught of harmful environmental mutagens. More broadly, variable selection on mutagen tolerance plays an important role in local mutation rate evolution.

290F **Mapping Genetic Variation in Mating Plug Ejection Timing in *Drosophila* Females** Jolie A Carlisle[1],

Rachel Miller[1], Mikaela Matera-Vatnick[1], Dawn Chen[2], Adriana Andrus[3], David Cabello[1], Andy G Clark[1], Mariana F Wolfner[11]Molecular Biology and Genetics, Cornell University, [2]Department of Biology, University of Pennsylvania, [3]Ohio Northern University

Males of internally-fertilizing species transfer seminal fluid proteins (Sfps) along with sperm to their mates. Sfps mediate essential reproductive changes in the female, such as increased egg production, ovulation, and female sperm storage, so through them males can exert influence over fertility. However, from mammals to invertebrates, females are known to also exert control over their reproductive outcomes. For example, *Drosophila*, females can influence the proportion of progeny they produce from two rival males, by controlling sperm storage and utilization. For both male and female *Drosophila*, the mating plug is a likely focal point for control of mating outcomes. The MP, which is composed of Sfps and female proteins that coagulate in the female's uterus (bursa), is thought to retain sperm, pheromones and SFPs, promoting fertility. Rapid ejection of the MP prevents sperm retention, while delayed ejection of the MP increases the number of sperm that can be stored. Thus, by controlling how long MPs are retained a female can potentially control the relative proportion of her progeny from rival males. By examining rates of MP ejection in females from 70 DGRP lines, mated to a standard male, we find dramatic differences (>2-fold) in the timing of mating plug ejection. Our GWAS has identified gene candidates that could mediate females' MP ejection timing. We are using knockdowns of candidates with high expression in female reproductive tissues or the nervous system to test for roles in MP ejection.

291F **Characterization of Female Meiotic Prometaphase I in *Drosophila ananassae*** Langston Pendleton, William D GillilandBiological Sciences, DePaul University

The Muller F element chromosome in *Drosophila ananassae* has evolved to become much larger than the homologous chromosomes from other species. Genomic analysis has found that this expansion is largely due to accumulation of heterochromatin. As heterochromatin forms the tethers connecting nonexchange chromosomes (such as the F element in *D. melanogaster),* this raises the question of what meiosis looks like in this species. A previous study from our group examined meiosis in several *Drosophila* species and found that chromosome size correlated with the average chromosome separation distance during prometaphase as well as the amount of time chromosomes spend out on the spindle during prometaphase. We are examining female meiosis in *D. ananassae* to test three hypotheses about this chromosome: First, we will measure the average separation distance between the F element chromosomes, and estimate the time spent out on the spindle based on the proportion of fixed images with separated prometaphase chromosomes. Second, we will use antibody preps to characterize the heterochromatin threads tethering these nonexchange chromosomes. Third, while population genetic analyses have estimated that the F element has a low rate of recombination, we will directly measure recombination on this chromosome using PCR and restriction digests.

292S **Intralocus sexual conflict drives new gene evolution in *Drosophila*** Deanna Arsala[1], Shengqian Xia[1], Andrea Gschwend[2], Shuaibo Han[3], Laura Faulere[4], Jared Atlas[5], Daniel J Sanchez[6], Manyuan Long[71]Ecology and Evolution, University of Chicago, [2]The Ohio State University, [3]Zhejiang University, [4]University of Latvia, [5]Committee on Genetics, Genomics, and Systems Biology, The University of Chicago, [6]University of Chicago, [7]Ecology and Evolution, The University of Chicago

Males and females of nearly all sexually reproducing species pursue divergent reproductive strategies to reach their fitness optima despite sharing the same genetic material. These differences can cause intralocus sexual conflict (ISC), where the presence of a shared genetic trait increases fitness in one sex while decreasing fitness in the other sex.

Theoretical and genetic association studies have suggested that ISC can be resolved through the modification of sex-specific gene expression or alternative splicing. However, we still have little empirical evidence of the genes involved in ISC and their direct impact on sex-specific fitness in evolution. A recent study from our laboratory showed for the first time that gene duplication can mitigate sexual conflict in *Drosophila melanogaster*. The generality of this case is unknown and presents an important problem.

We set out to understand whether intralocus sexual conflict can generally drive the evolution of new genes in *Drosophila*. Using a combination of CRISPR/Cas9, constitutive and tissue-specific RNAi knockdowns, we have examined the fertility of 125 evolutionarily young and sex-biased genes in *Drosophila*. This ongoing experiment has revealed so far that one-third of young genes have roles in sexual conflict, contrary to the initial expectation that nearly all should be adaptive for males and females. Further functional analysis revealed that these genes are involved in sex conflict in reproductive tissues and in a unidirectional pattern, where they benefit male fertility at the expense of female fertility. Our preliminary data suggest ISC may be a general driver of new gene evolution.

293S **The evolution of ovary-biased gene expression in Hawaiian *Drosophila*** Samuel H Church[1], Catriona Munro[2], Casey Dunn[3], Cassandra Extavour[41]Ecology and Evolutionary Biology, Yale University, [2]Collège de France, [3]Yale University, [4]Harvard University

How well do expression atlases from model organisms predict patterns of expression in other species? To answer this question, we need robust hypotheses that describe how much variation in gene expression we expect to observe over evolutionary time. Here we provide an answer by analyzing RNAseq data across twelve species of Hawaiian Drosophilidae flies, focusing on gene expression differences between the ovary and other tissues. We show that there exists a cohort of ovary-specific genes that is stable and that largely corresponds to described expression patterns from laboratory model *Drosophila* species. We also show that, as the evolutionary distance between species being compared increases, variation between species overwhelms variation across tissues. We reconstruct the evolutionary history of genes that have changed expression patterns, and identify genes that have gained or lost expression in the ovary. We then use the dataset of evolutionary changes in expression to test for signatures of correlated expression evolution between genes. We show that genes that are predicted to interact show more correlated evolution than we expect by chance. Finally, we use the correlation data to generate new hypotheses about networks of genes that share evolutionary trajectories.

294S **Disentangling the linkage between an ecological trait and the corresponding mate preference** Wei Lu[1], Marcus Kronforst[21]Ecology and Evolution, University of Chicago, [2]University of Chicago

Speciation, the formation of new species, is a key process that generates present-day biodiversity patterns. The evolution and maintenance of new species usually requires both prezygotic isolation and postzygotic isolation mechanisms. Many speciation genetics studies have focused on the genes that cause hybrid inviability or sterility (postzygotic isolation mechanisms). We still know little about genes causing prezygotic isolation, such as assortative mating. Extensive color pattern variations exist across the *Heliconius cydno* species range and males exhibit strong color-based mate preference. Previous GWAS for male mate preference in *Heliconius cydno* identifies a major effect preference locus. In accordance with theoretical predictions and several preference QTL mapping studies in other systems, this preference locus is in high linkage disequilibrium (LD) with the color locus. Both pleiotropy and suppressed recombination can create strong LD patterns. To investigate the role of structural variants (SV) in suppressing local recombination, we generate Nanopore long-read sequencing data for multiple *Heliconius cydno* individuals and compare the SV distributions in the focal region. We also map the male mate preference in a different population with low LD between the trait locus and the preference locus to understand the pleiotropic effect.

295S **Investigating the relationship between compensatory gene regulatory evolution and gene misexpression in Drosophila hybrids** Sophie Ross, Manaal Fatima, Garrett Larivee, Joseph CoolonWesleyan University

Evolution is fundamentally a tug of war between forces that change and maintain organismal form and function. The evolutionary process faces a challenge in that most phenotypes must be stably maintained in the presence of new mutations, yet organisms must retain the capacity for phenotypic change that allows them to adapt to new environments. Evolutionary biologists often focus on these changes, but the genetic mechanisms and evolutionary processes maintaining phenotypes are equally important, in part, because they maintain function while allowing hidden genetic variation to accumulate with the potential to cause misexpression of genes upon species hybridization. Comparative studies of gene expression and gene regulation among closely related strains and species of diverse organisms suggest that compensatory changes in the gene regulatory networks controlling gene expression often result in conserved phenotypes despite changes in the underlying DNA sequence. However, the contribution of compensatory cis- and trans-regulatory changes to gene misexpression observed with greater evolutionary distances remains unknown in part due to differences in tissue abundance in the whole organisms used for these analyses in Drosophila to date. Here we measure gene expression and regulatory change genome-wide in a series of comparisons between Drosophila species with increasing levels of evolutionary divergence from 10,000 years to 2.5 million years using RNA-seq. Using head tissues, we have minimized the consequences of differences in tissue abundance observed in sterile hybrids between more distant species allowing for testing the association between compensatory cis-trans regulatory change and gene misexpression in hybrids.

296S **Functional consequences of the rapid evolution of a putative *de novo* evolved gene required for sperm function in *D. melanogaster*** Salim J Metri, Brenna K McCormick, Prajal H Patel, Geoffrey D FindlayBiology, College of the Holy Cross

De novo evolved genes emerge from previously non-coding regions of DNA and are frequently expressed in the male germline. We conducted an RNAi screen to identify putative *de novo* genes in *D. melanogaster* that are required for male fertility. When one such gene, *saturn*, is knocked down or knocked out, male fertility is severely reduced for at least two reasons: a significant reduction in sperm production, and impairment in the ability of transferred sperm to migrate to the site of fertilization in females. To investigate the cellular localization and function of Saturn protein, we developed an antibody to Saturn and an HA-tagged rescue construct. Cytological investigation with these tools revealed that Saturn localizes to the nuclear periphery during post-meiotic stages of sperm nuclear condensation. In *saturn* null males, nuclear bundles become misshapen at the late canoe stage of condensation. Because we found previously that Saturn has evolved under positive selection within the *melanogaster* group of *Drosophila*, we next investigated whether these protein sequence changes affect Saturn's protein localization and contributions to fertility. By expressing codon-optimized, HA-tagged orthologs of *saturn* in *D. melanogaster* males lacking endogenous copies of the gene, we found that the *D. yakuba* ortholog only partially restored fertility, while the *D. ananassae* ortholog was completely unable to rescue fertility. We observed that the *D. ananassae* ortholog mislocalized in *D. melanogaster* testes, consistent with either extreme functional divergence between these species or lineage-specific coevolution with interacting partners. We are currently testing inferred ancestral orthologs to pinpoint the timing with which Saturn gained its specific localization pattern and function in *D. melanogaster*. In parallel, we are analyzing mutant forms of the *D. melanogaster* gene to identify specific residues necessary for Saturn's localization and function. Taken together, these data advance our understanding of how novel genes evolve essential biological functions.

297S **Molecular basis of gene co-option in mimicry phenotype evolution** Sofia I Sheikh, Nicholas W VanKuren, Meredith M Doellman, Marcus R KronforstEcology and Evolution, University of Chicago

Novel phenotypic diversity is frequently generated via small tweaks or re-deployment of ancient and important genes rather than the evolution of new ones. However, the functional basis of how these genes gain novel functions while retaining their ancestral one remains an understudied question. In particular, little is known about how co-opted genes are regulated in these secondary contexts, how they regulate downstream targets, and whether existing or novel GRNs are involved in producing the novel phenotype. I aim to shed light on this question through an investigation of a classic case of gene co-option: *doublesex*-mediated mimicry in swallowtail butterflies (*Papilio*). *Doublesex* (*dsx*) is the master regulator of sex differentiation in insects but has been co-opted for the evolution of Batesian mimicry. In this novel context, allelic variation at *dsx* determines female wing color patterning and results in co-occurring mimetic and non-mimetic female morphs across five *Papilio* species. While the female-limited mimicry polymorphism has been traced back to *dsx* in all five species, the mimetic alleles in each are highly divergent, making it unclear whether this represents a case of ancestral or convergent co-option, and thus whether the mimicry switch is mediated via the same processes and pathways. I am leveraging this system for a comparative study across these species to decipher shared and unique evolutionary and functional aspects of co-option. Specifically, I will compare sequence and structural variants both between mimetic and non-mimetic alleles within each species, as well across species. Additionally, I will characterize the functional basis of *dsx*-mediated mimicry using immunostaining and TF pull-down experiments. Finally, candidate CREs can be identified from these datasets and experimentally validated using CRISPR knock-ins and knock-outs. Altogether, this work will shed light on how co-option of existing genes and pathways can enable the evolution of phenotypic adaptations.

298S **Contribution of chitin-related genes to octanoic acid resistance in *Drosophila sechellia*** Katherine Fhu, Vivian Lu, Joseph CoolonBiology, Wesleyan University

Drosophila sechellia is a dietary specialist species of fruit fly, native to the Seychelles Islands where it feeds almost exclusively on the fruit of *Morinda citrifolia*, commonly known as noni fruit. Ripe noni fruit contains volatile toxins, primarily octanoic acid (OA) and hexanoic acid (HA), as a lethal method of defense that works against herbivorous insects, including *D. sechellia*'s generalist sister species. The genetic and molecular mechanisms of *D. sechellia*'s resistance to OA and HA remain unclear. Previous genetic screens have shown that several genes are differentially expressed in *D. sechellia* upon exposure to OA and HA (including *Peritrophin-15a*, *Gasp*, *Chitinase 7*, and *Chitinase 9*). We tested chitin-related genes by knocking down each with RNAi in *D. melanogaster* to decrease their expression, and survival assays were conducted to observe changes in the flies' ability to survive exposure to OA. Our analysis revealed that *Chitinase 7* may play a role in OA resistance, making it a promising candidate for further investigation.

299S **Causes and consequences of *doublesex* co-option in the evolution female-limited color pattern polymorphism** Nicholas VanKuren[1,2], Meredith M Doellman[3], Sofia I Sheikh[3], Daniela H Palmer Droguett[3,4], Darli

Massardo[3], Marcus R Kronforst[31]Ecology & Evolution, University of Chicago, [2]The University of Chicago, [3]Ecology & Evolution, The University of Chicago, [4]Biology, University of Texas at Arlington

Novel phenotypes are increasingly recognized to have evolved by co-option of genes into new developmental contexts, yet the changes induced by co-option remain obscure. Here we provide deep insight into this process by characterizing the consequences of *doublesex* co-option in the evolution of mimetic wing color patterns in *Papilio* swallowtail butterflies. *doublesex* is the master regulator of insect sex differentiation, but novel *dsx* alleles control the switch between discrete female wing color patterns in *Papilio polytes*. We show that a pulse of widespread *dsx* expression early in mimetic wing development activates an alternate color pattern development program that becomes decoupled from *dsx* expression itself. RNAi and antibody stains showed that Wnt signaling antagonizes *dsx* function in some regions of the wing to refine the mimetic color pattern, but that *dsx* function depends on *engrailed,* the key effector of the Hedgehog signaling pathway. Dsx alters spatial patterns of En expression early in pupal wing development, but the two genes quickly become decoupled by mid-pupal development when En expression pre-figures melanic and red patterns in all *P. polytes*. We conclude that co-option of *dsx* into the wing development network results in global changes to color patterning programs that function to antagonize and synergize with *dsx* to specify a novel adaptive phenotype. Current functional genomics analyses shed light on the causes of differential expression of *dsx* alleles and the evolutionary process by which *dsx* was co-opted. Altogether, our findings provide strong experimental evidence for how co-opted genes cause and elicit changes to gene regulatory networks during the evolution and development of novel phenotypes.

300S **Evolution of a fatty acyl-CoA elongase underlies desert adaptation in *Drosophila*** Zinan Wang[1], Henry Chung[21]Department of Entomology; Ecology, Evolution, and Behavior program, Michigan State University, [2]Michigan State University

Adaptation to diverse and extreme environments is key to long-term species persistence. One of the largest challenges for organisms living in terrestrial environments is water loss. Insects use cuticular hydrocarbons (CHCs), a lipid layer on the body surface, to reduce water from evaporation and therefore, withstand desiccation stress. It has long been hypothesized that the waterproofing capability of this CHC layer, which can confer different levels of desiccation resistance, depends on its chemical composition. **However, it is unknown how the evolution of CHC components determine differences in desiccation resistance in insects and how insect species can evolve high levels of desiccation resistance.** In this study, we determined desiccation resistance and CHC compositions across 50 *Drosophila* and related species We showed that the length variation in a subset of these CHCs, the methyl-branched CHCs (mbCHCs), is a key determinant of desiccation resistance in *Drosophila*. In particular, the evolution of longer mbCHCs underlies the evolution of higher desiccation resistance. We identified a fatty acyl-*CoA* elongase gene in *D. melanogaster* and *D. mojavensis*, which we named *mElo*, responsible for the elongation of mbCHCs. We showed that the overexpression of *Dmoj/mElo* in *D. melanogaster* led to longer chain mbCHCs and significantly higher desiccation resistance. In addition, CRISPR/Cas9 knockout of *Dmoj/mElo* in *D. mojavensis* led to loss of longer mbCHC production and significant reduction of desiccation resistance at a temperature relevant to desert environments. Phylogenetic analysis showed that the *mElo* gene is specific to the *Drosophila* genus, suggesting a lineage specific adaptation mechanism.

301S **How do the bodies of larval insects evolve?** Bianca Campagnari[1], Samuel H Church[2], Bruno AS de Medeiros[3], Sam Swank[4], Annika Sharma[4], Ellie Heckscher[1], Seth Donoughe[11]MGCB, University of Chicago, [2]Yale, [3]Field Museum, [4]University of Chicago

Over evolution, there is a remarkable diversity in animal body forms. A vivid example is the body forms of adult insects, some of which have no wings, two wings, or four wings. Two main drivers of diversity in body form are phylogeny and habitat. To probe the effects of these drivers, we use as a model larval insects in the Dipteran order. The Dipteran order spans about 250 million years of evolution, is very diverse, and contains more than 150,000 described species. Its taxonomy is established. Almost all Diptera undergo metamorphosis, with different larval and adult body forms that are highly adapted to their specific habitats. Thus, we have ample species to choose from for comparative work. Importantly, the order includes the well-studied model, *Drosophila melanogaster,* which serves as a point of reference for this study and future studies. Here, we assembled a novel dataset that integrates body form information, habitat and phylogeny. We conduced an extensive literature review scoring traits of over 50 families and 700 genera within Diptera. We especially extensively sampled clades that were identified as nodes of transitions in habitat that are potentially relevant to the movement and therefore body form (e.g.: transitions between totally aquatic living, endoparasitism, and leaf mining). In terms of body form, we scored size and shape. Where possible, we scored larval size at each instar (stage), proleg presence, presence of cuticular protrusions, and body decorations thought to be relevant to locomotion

such as creeping welts. In addition, we used barcode DNA sequences to create an intra-Diptera phylogenomic tree. First, we report on the surprising morphological diversity in larval body size and decoration. Second, we will report on the relationships between body features, habitat, and phylogeny. We hope to determine ancestral states of larval traits and how these features have co-evolved with shifts in larval ecology. Together it is expected to provide novel insight into the evolution of diverse body forms. It will stage for systematic interrogation of underlying neuromuscular systems that control the movement of the diversity of body forms.

302S **Nucleotide-level precision in transcript distance (*TranD*) metrics: comparison of *D. melanogaster* and *D. simulans* transcriptomes** Adalena Nanni[1], James Titus-McQuillan[2], Oleksandr Moskalenko[1], Francisco Pardo-Palacios[3], Sarah Signor[4], Srna Vlaho[5], Rebekah Rogers[2], Ana Conesa[6], Lauren McIntyre[11]University of Florida, [2]University of North Carolina, [3]Polytechnical University of Valencia, [4]North Dakota State University, [5]Charles River Laboratories, [6]Institute for Integrative Systems Biology, Spanish National Research Council (CSIC)

The sister species, *D. melanogaster* and *D. simulans* have diverged relatively recently (~5 million years ago). *Drosophila melanogaster* has an exceptionally well annotated genome, and the species pair is a model for comparative genomic approaches. Gene annotations and exon identification across species is a mature field, but little attention has been paid to identifying transcript orthologs. Being able to quantitatively compare transcript models within and between species is an important first step toward understanding questions about evolution of transcript structure. We propose a set of distance metrics using nucleotide-level quantification of structural phenotypes (intron retention, donor/acceptor variation, alternative exon cassettes, alternative 5'/3' UTRs). We compared annotated transcript models between *D. melanogaster* and *D. simulans a*nd created an index that links transcript between species. Of the 16,009 genes that map to both species, 94% of the 12,386 annotated one to one orthologs have reciprocally matching transcripts with identical junctions, while 5% contain exons annotated in only one species. In an RNA-seq experiment that contains both long and short reads from head tissue of both species we found support in the data for exons annotated in the other species. We further found support in both species for reciprocally matching transcripts more broadly. Distance metrics are implemented in *TranD,* a PyPi package. We demonstrate the general utility of these metrics by i) comparing transcript models from different bioinformatic tools such as IsoSeq3 and FLAIR ii) describing the annotated transcriptomes of several species iii) comparing long read data to annotated transcript models.

303S **A mitonuclear reality check on the evolutionary significance of Mother's Curse in Drosophila** David M Rand, Faye M Lemieux, Kenneth M Bradley, Lindsay M MarmorDepartment of Ecology, Evolution and Organismal Biology, Brown University

Maternal inheritance allows selection to act on mtDNA-encoded effects in females, but prevents direct selection on mtDNA in males. Mutations that are deleterious in males but neutral or beneficial in females can persist in populations. This predicts that mtDNA-based disease or phenotypic variation should be more common in males, while haploid selection in females will purge mtDNA-based variation (Frank and Hurst (1996); repackaged as the 'Mother's Curse' by Gemmell et al. (2004)). There is conflicting evidence for this pattern in the literature. A key assumption in Mother's Curse is that mtDNA phenotypes must be sex limited with different effects, even different signs, in males and females. Extreme Mother's Curse scenarios invoke mtDNA mutations that are beneficial in females and deleterious in males and sweep through populations leading to extinction from male unfitness. Comparisons of sex-specific mtDNA phenotypic effects from different populations and species are needed to evaluate the evolutionary significance of Mother's Curse.

Most Mother's Curse analyses use alternative mtDNAs placed on one or more homozygous nuclear chromosomal backgrounds. Since most organisms are heterozygous at many loci, we sought to perform experiments in several different heterozygous backgrounds. MtDNAs from Drosophila melanogaster (OreR and Zimbabwe), D. simulans (siI and siII) and D. yakuba were each placed on a common D. melanogaster w1118 nuclear background. Virgin females from these strains were crossed to males from each of several deficiency stocks carrying a hemizygous segment of chromosome 2L. F1 female and male flies carrying the deficiency chromosome and the w1118 chromosomes were tested for starvation, climbing and flight performance. For all three traits in the majority of chromosomal backgrounds, the variance among mtDNA genotypes was greater in females than in males. This result is the opposite of the Mother's Curse prediction. Moreover, the impact of the foreign D. yakuba mtDNA was equally neutral or beneficial in both males and females, suggesting some form of phylogenetic heterosis. The mitonuclear epistatic interactions across the different heterozygous backgrounds and the five mtDNA haplotypes are more pronounced in females than males. This suggests that mtDNA interactions with regional hemizygosity or dominance effects are more pronounced in females than males, overshadowing any effect of Mother's Curse or even hidden Y chromosome variation.

304S **A genetic and comparative approach to uncovering the evolution of inter-cellular lipid transport in animals.** Bryan T Rogers[1], Md. Ehtear Hossain[21]Biological Sciences and Chemistry, Southern University and A & M, Baton Rouge, [2]Southern University

We are using simultaneous exploration in flies and mice to discover the evolution of lipid transport and management in the evolution of animals. We have begun with genetic investigations of the fruit fly into phenomenon characterized in mouse and human tissue culture systems. We intend to find examples where inter-cellular lipid transport regulation is shared between the two species, suggesting broad conservation across animals and revealing critical functions of lipids and lipid metabolism. Also, examples of disparate or strictly analogous functions would suggest adaptive changes during fly and mouse evolution. We have begun by investigating inducible changes in lipid absorption mediated by lipid scavenger receptors in the mouse.

305S **Annotation of Insulin-like protein 8 in *D. persimilis* and *D. miranda*** Adriana Andrus, Jamie SidersSchool of Science, Technology, and Mathematics, Ohio Northern University

The Pathways Project is a Genomics Education Partnership (GEP) annotation project focused on the annotation of Drosophila genes in the insulin signaling pathway across 27 species as a means of unraveling the evolution of complex biological networks. The insulin pathway is critical to organismal growth and metabolic homeostasis, and is conserved among various species of Drosophila. This research concentrated on the annotation of Insulin-like protein 8 (Ilp8) in both Drosophila persimilis (D.per) and Drosophila miranda (D.mir). In Drosophila melanogaster (D.mel), the Ilp8 gene has one isoform, Ilp8-PB, with three exons located on the negative strand of chromosome 3. While Ilp8 possesses a paralogous evolutionary relationship to other Insulin-like proteins that bind to insulin receptor (InR), Ilp8 is not known to be directly involved in the pathway. Prior to annotation, it was hypothesized that there would be moderate to high conservation between Ilp8 in D.mir and D.per as they are not significantly diverged species, sharing a recent common ancestor (2 MYA). Data from examination of the genomic neighborhoods from both species provided evidence of synteny; upstream genes beg and scaf6 and downstream genes Fit2 and CG7730 were conserved across D.per, D.mir and the reference D.mel genomes. Manual annotation of the coding regions of Ilp8 in both D.per and D.mir and subsequent protein alignment to D.mel revealed relatively high levels of conservation. BLASTp alignment of the D.mir Ilp8 to the reference D.mel sequence showed 75% similarity while BLASTp alignment of D.per to D.mel showed 78% similarity. Future work will include annotation of the coding sequences of additional Ilp8 orthologs across more diverged Drosophila species and comparison of Ilp8 evolutionary rates to that of genes in the canonical insulin signaling network. Given that Ilp8 is not directly involved in the insulin signaling pathway, it is predicted that it will evolve at a faster rate than paralogous genes with higher degrees of connectivity within the pathway. Following annotation of the coding regions of genes in this biological network, annotation and analysis of regulatory regions will be conducted to advance our understanding of the evolutionary constraints placed on regulatory regions in comparison to coding regions of genes in signaling pathways.

306S **Dynamic evolutionary history of DNA-protein crosslink repair proteins in *Drosophila*** Cara Brand, Mia LevineUniversity of Pennsylvania

DNA damage poses a chronic threat to genome integrity. Decades of research on DNA repair mechanisms have revealed how non-homologous end joining, base excision repair, homologous recombination, and other pathways preserve the fidelity of genetic inheritance. Recently, a new type of repair pathway has been identified. Proteins that perform various DNA transactions transiently crosslink with the DNA. However, mutagens or enzymatic errors can trap proteins in permanent crosslinks. Irreversible DNA-protein crosslinks (DPCs) obstruct essential cellular process like replication and chromatin remodeling. The recently identified Spartan family of proteases degrade the protein component of these deleterious DPCs. Spartan family proteases are ubiquitous across eukaryotes. However, we recently found that the Spartan protease, MH, evolves under positive selection between *Drosophila melanogaster* and its close relative *D. simulans*. We experimentally demonstrated that MH evolves adaptively to mitigate DNA damage triggered by a rapidly evolving *D. melanogaster*-specific DNA satellite array. The ubiquitous abundance and remarkable rapid evolution of DNA satellites raises the possibility that MH coevolution with a satellite DNA is not limited to *D. melanogaster*. Instead, Spartan family innovation might be pervasive across the broader *Drosophila*phylogeny. To explore this possibility, we conducted tests of molecular evolution and phylogenomic analyses using the recently published, highly contiguous 101 *Drosophila* species genome assemblies. We found that multiple Spartan family genes evolve adaptively across diverse evolutionary timescales: between closely related species as well as across clades spanning ~10 million years of evolution. Using BLAST, synteny analysis, and phylogenetic tree building across 48 *Drosophila* genomes, we revealed that three Spartan family genes are retained across the *Drosophila*phylogeny. Remarkably, we also discovered that one Spartan

protease recurrently duplicates, birthing 13 young paralogs. Finally, we documented numerous evolutionary transitions between ovary- and testis-biased expression across Spartan family orthologs and across parent and daughter paralogs. This discovery raises the novel and unexplored idea that the male and female germlines have distinct requirements for DPC repair. We are now probing the drivers of Spartan family diversification, with a focus on DNA satellite evolution. Our findings are poised to reveal the genetic causes and functional consequences of innovation at DPC repair proteins in *Drosophila* and beyond.

307S **Gene expression evolution in the Drosophila female somatic reproductive tract** Rachel C Thayer, Elizabeth Polston, Giovanni Hanna, David BegunEcology & Evolution, University of California, Davis

The somatic tissues in the Drosophila female reproductive tract have been understudied relative to male tissues, and major questions about their function and evolution stand unsolved. Here, we present a single nucleus gene expression atlas of the seminal receptacle, spermatheca, female accessory glands, oviduct, bursa, and reproductive-associated fat body, with cell types verified using in situ hybridization. These tissues were not targeted or annotated in the recent Fly Cell Atlas consortium, and only the spermatheca is represented in FlyAtlas 2. With these data, we identify spatially distinct regions within the seminal receptacle, evolutionarily conserved marker genes for each cell type, and functional gene enrichments within cell types. Next, we investigated the tempo of gene expression evolution and the extent of male-female coevolution in these tissues. Recent work found striking gene expression evolution in the male tissues that produce seminal fluid between high and low latitudes in the classic North American cline. In particular, there was a significant enrichment for reduced seminal fluid protein expression in the low latitude population, with evidence for recent divergence and positive selection. We hypothesized that these evolved differences may be driven by sexual conflict, which would predict strong co-evolutionary dynamics in females. To investigate female-ejaculate co-evolution, we measured post-mating gene expression in female tissues that are directly exposed to ejaculate, after mating to either a same-population or a diverged partner. If females and males are co-adapted, then mismatched matings should show aberrant gene expression. We find little evidence of female-ejaculate co-evolution, with no effect of male origin on female post-mating gene expression. Rather, we found a robust, highly conserved post-mating response, in which more than 4000 genes are differentially expressed after mating (i.e. more than half of the reproductive tract transcriptome).

308S **Abdominal pigmentation in the *Drosophila montium* species subgroup as a model for investigating the molecular basis of sex-limited polymorphisms and the evolution of dominance.** Yuichi Fukutomi[1,2], Emily K Delaney[1], Masayoshi Watada[2,3], Artyom Kopp[11]UC Davis, [2]Tokyo Metropolitan University, [3]Ehime University

Sex-limited polymorphisms are found in diverse animal species. While many theoretical models and empirical studies have sought to elucidate the evolutionary forces responsible for their origin and maintenance, the molecular mechanisms that specify sex-limited polymorphisms are poorly understood. Similarly, extensive population-genetic theory has been developed to explain the evolution of dominance, but the mechanistic basis of dominance reversals remains unknown. Abdominal pigmentation in the *Drosophila montium* species subgroup provides an excellent model for investigating both these questions. Many species in this clade display a Mendelian, female-limited color dimorphism (FLCD). Genome-wide association studies reveal that independent regulatory mutations in the *POU domain motif 3* (*pdm3*) gene, which acts as a repressor of pigmentation, are responsible for the repeated evolution of FLCD in multiple *montium* subgroup species. However, antibody staining shows that Pdm3 is expressed in both dark and light genotypes, 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 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.

309S **Genetic mechanisms underlying temperature preference adaptation in *Drosophila melanogaster* from Africa and Europe**

José Miguel Simões[1], Tiago da Silva Ribeiro[2,3], Stephanie M Kim[1], Peixiong Zhao[1], Courtney Rawitscher[1], Annaliese Krausse[1], Gammachu Amae[1], Alessia Para[1], Marcus Stensmyr[4], John E Pool[2], Marco Gallio[11]Neurobiology, Northwestern University, [2]Integrative Biology, University of Wisconsin-Madison, [3]Laboratory of Genetics, University of Wisconsin-Madison, [4]Biology, Lund University

The common fruit fly *Drosophila melanogaster* originates in Southern Africa but has expanded to colonize nearly all temperate regions of the world initially following human migrations and later as a carry-on on commercial routes. The ability to adapt to new thermal environments has been a key process in allowing this small poikilotherm to colonize new habitats. In general, temperature adaptation involves changes in biochemistry and physiology that allow an animal to function in a new thermal environment. In parallel, temperature preference also changes, so that the animal eventually prefers a different thermal range, matching the conditions that best support its fitness in the new environment. How temperature preference evolves during the colonization of new environments in animals is not known. To address this question we first tested several wild-derived fly strains collected in various locations along the “out of Africa” migration (from Zambia to France) and observed that, in rapid laboratory assays for thermal preference (2-choice tests), they display systematic differences in temperature preference, with strains from France and Zambia showing the most significant difference. Next, our goal has been to identify the genetic variation behind this shift in temperature preference. For this, we used a mapping approach involving battery of 308 unique recombinant inbred lines (RILs) obtained from crosses between a Zambian and a French parental population. Our preliminary results suggest a complex genetic architecture behind the evolution of temperature preference in these strains, but also point to key candidate loci as a starting point to further experimental work that uses genetic manipulation, functional imaging and circuit studies in laboratory strains of *D. melanogaster*. Our hope is that this work will eventually lead to a better mechanistic understanding of the genetic processes that drive the evolution of thermal preference in *Drosophila*, and more in general, contribute to our understanding of how behavior evolves under changing environmental conditions.

310S **Cryptic suppression reveals intragenomic conflict in the *Sex Ratio* system of *Drosophila pseudoobscura***

Jackson Bladen, HJ Nam, Spencer Koury, Graham Taggert, Nitin PhadnisUniversity of Utah

Sex Ratio (*SR*) chromosomes are selfish *X* chromosomes that undermine the transmission of Y chromosomes 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 rare recombinant chromosomes that reflect the ancestral state of the *SR* chromosome in North American populations. In segregation assays using these rare 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.”

311S **Identification of degenerate 9+0 and 9+2 axoneme motifs in the *Drosophila* major B1 and testis-specific B2 tubulins** Kati Bowsher, Olivia Parson, MARK GORDON NIELSENUniversity of Dayton

How does a protein evolve while maintaining its function? Structure/function tests of the spermtail protein B2-tubulin show that even small changes in the protein render it unable to generate a motile spermtail, raising the question about how it evolved in the first place. In fact, it has not evolved in 60 million years in *Drosophila melanogaster* and its relatives. In previous work, we identified additive and synergistic amino acid specializations of the B2 protein. Synergism is of particular interest because it makes evolution path-dependent, slowing its progress and potentially contributing the 60 million-year stasis of the B2 protein. Two amino acids, Thr55 and Ala57, were identified as potentially participating in a B2-specific synergism, their function depends on amino acid contacts that are only present in B2, and their amino acid identities are unique to B2 in comparison to other beta tubulins. Here we test the hypothesis that a third amino acid that is in contact with Thr55 and Ala57 in the folded protein and is also unique to B2, Cystein29, completes the synergism, by generating transgenic flies (called "CTA") expressing a modified major B1 tubulin containing B2 identity Cys29, Thr55 and Ala57.

Spermatogenesis and fertility were assessed in CTA/CTA B2null/B2null flies. CTA is able to support all post-mitotic tubulin function in the testis (cell division and shaping), except spermatogenesis. CTA flies generate short, immotile sperm and are sterile. No additional sperm-generating function was provided by Cystein29 compared to the B1-B2 construct containing only Thr55 and Ala57.

There is not a clear candidate for sperm generating function in the 11 remaining differences between CTA and B2, indicating that sperm generating function does not depend on a particular motif but resides in small epistatic interactions throughout the protein. However, the lack of strong candidates for remaining B2 function raises another possibility, that the B1 backbone we used in testing for B2 amino acid specializations is not neutral, but contains 9+0 specializations that actively generate immotile axonemes, putatively in support of its role in generating a non-motile, 9+0 axoneme in the chortodonal organs of the fly. Candidates for such B1 specializations will be identified though bioinformatic comparisons among 9+2 and 9+0 axoneme-generating beta tubulins and tested in transgenic flies.

312S **Limits of adaptation in a sperm-generating protein: evolution along a narrow path in Drosophilid B2 tubulin** Olivia Parson[1], Kati Bowsher[1], Rihanna Domingos[2], MARK NIELSEN[11]University of Dayton, [2]University Of Dayton

Drosophilid spermtails are the peacock feathers of the world of sperm by virtue of their incredible length, from 2mm in *Drosophila melanogaster* to over 5cm in *D. bifurca*. *D. melanogaster* use a specialized, testis-specific B2 tubulin isoform to generate the microtubule scaffolding of their spermtail axoneme. Structure/function tests show B2 exerts considerable control over sperm morphology, from 9+2 ultrastructure to length. Insect sperm are subject to strong sexual selection and it may be expected that B2 plays an important role in gamete evolution. However, structure/function tests show B2 does not tolerate change, even small alterations in its amino acid sequence render it non-functional. This sensitivity is reflected in its evolutionary stasis, the Drosophilid B2 protein has not evolved at a single amino acid in 60 million years.

There are two hypotheses for its stasis, either 1) the DmB2 protein is an ideal configuration that has outcompeted variants over the past 60 million years, or 2) it is the only configuration able to support the Drosophilid sperm, it evolved into a corner it cannot evolve out of that is resistant to evolutionary change and templates long axonemes. These are tested by assessing the ability of *Glossina morsitans* GmB2, 96% identical to DmB2, and the *Homo sapiens* sperm generating beta tubulin HsB3, 90% identical to DmB2, to replace DmB2 function. Tsetse fly B2 is able to support spermatogenesis and fertility in transgenic DmB2 null flies, it generates long, fertile sperm as assessed by light microscopy on testis preparations and fertility tests in crosses between transgenic males and virgin females. This supports the first hypothesis that there are other versions of B2 able to support spermatogenesis. This provides to potential for B2 to participate in the adaptive process, for example through allelic effects on sperm length, an important factor in sperm retention in the female reproductive tract.

The human sperm beta tubulin generates sperm in *Drosophila* but does not support fertility, indicating there are both generic and lineage-specific requirements for sperm beta tubulin function. The ability of the sperm-generating beta tubulins to support some amount of spermatogenesis contrasts to a B1-B2 chimeric protein "CTA" that shares greater identity (97.5%) to DmB2 yet does not support spermatogenesis. This indicates the presence of either a degenerate sperm-generating motif shared by the testis beta tubulins, or a sperm-inhibiting motif in the major B1 protein used in testing B2 amino acid specializations. Identification of these potential motifs will follow through bioinformatic

approaches to identify candidates followed by testing in transgenic flies.

313S **The viability and segmentation defects of heterozygotes for two *even-skipped* (eve) lethal mutations are vastly different.** Michael Z LudwigEcology and Evolution, University of Chicago

I address a fundamental biological question: How do lethal mutants affect fitness when carried over a “normal” allele? Two ***eve*** lethal mutants of *D. melanogaster* were tested for relative viability in heterozygous state: ***eve*** ^{R13} is a classic coding region lethal mutant; ***eve*** $^{\Delta MSE}$ is a recombinant **cis**-regulatory lethal mutant. To generate ***eve*** $^{\Delta MSE}$ I replaced the 480-bp Minimal Stipe Element (MSE) of the ***eve*** stripe 2 enhancer with *mini-white*$^{+}$ gene. The relative viability of flies carrying each ***eve*** lethal allele in heterozygote over a “normal” ***eve*** locus (HET) was compared to homozygous flies with two “normal” ***eve*** alleles, (viability of 1). The relative viability of females and males ***eve*** ^{R13} HET was, correspondingly, **0.8** and **0.65**, while for females and males of ***eve*** $^{\Delta MSE}$ HET it was **1.0** and **1.04**. This result shows that the lethal effect of the REGULATORY ***eve*** mutant in heterozygote is fully compensated with a “normal” ***eve*** allele, while for the CODING region mutant such compensation is only partial.

After demonstrating the difference in viability of ***eve*** ^{R13} and ***eve*** $^{\Delta MSE}$ HETs, I proceeded to investigate whether specific defects in segmentation could be observed. ***Eve*** stripe 2 corresponds to parasegment 3 in later stage embryos, therefore the size of parasegment 3 is a sensitive test for the ***eve*** function in stripe 2 area. Since parasegment 3 is bordered by ***engrailed*** stripes 3 and 4, the difference between **WT** homozygotes and the lethal heterozygotes in **Engrailed** pattern was evaluated as a ratio of parasegment 3 length to the sum of the lengths of parasegments 3 and 4 in stage 10 and 11 embryos. The ***eve*** ^{R13} and ***eve*** $^{\Delta MSE}$ HET embryos were identified by PCR genotyping. Ratios of parasegment 3 length to parasegments 3+4 length for **WT**, ***eve*** $^{\Delta MSE}$, and ***eve*** ^{R13} were correspondingly **0.5**, **0.47**, and **0.45**, and the differences between all three genotypes were statistically significant.

I conclude that HETs for the coding region mutant ***eve*** ^{R13} and the **cis-**regulatory mutant ***eve*** $^{\Delta MSE}$ demonstrate different impact on viability and on embryonic segmentation. Based on these results I predict that, some **cis**-regulatory mutants (including the deleterious ones) may exist in populations for many generations, before possibly encountering an epistatic interaction with a compensatory mutation at a different site in the genome.

314S **Gene Annotation of Akt in *Drosophila persimilis*** Julia Kaniuk, Jennifer MierischLoyola University Chicago

Examining gene conversation across many species is an important tool for observing divergence patterns in genes involved in vital cellular processes. This project contributes to the Genomics Education Partnership (GEP) Pathways Project whose main focus is examining the insulin signaling pathway, a key pathway in mediating glucose metabolism. The main goal of this project is to determine how well members of the pathway are conserved across *Drosophila* species and if changes have occurred, which molecules are affected and how consistent changes are across species. Existing evidence suggests molecules that act earlier in insulin signaling evolve more quickly than those acting later. *Drosophila persimilis*, the species investigated in this annotation, is a sister species of *Drosophila pseudoobscura* within the *obscura* group which diverged from the *melanogaster* groups beginning about 25 million years ago. This distant phylogenetic relation may imply an increased amount of divergence between *D. melanogaster* and *D. persimilis*. *D. melanogaster* has a well-characterized insulin pathway and therefore serves as a good reference point for *Drosophila* gene annotations. Using synteny to *D. melanogaster*, sequencing data from *D. persimilis*, and the UCSC Genome Browser, we were able to identify the genomic neighborhood of Akt in *D. persimilis*. Using BLAST and manual investigation, we were able to find and map the likely location of Akt in *D. persimilis*. We are currently investigating conservation of Akt in additional *Drosophila* species.

315V **Evolutionary diversification and repeated gene capture by telomeric retrotransposons across the *Drosophila* genus** Jae Hak Son[1], Mia T Levine[2], Christopher E Ellison[11]Genetics, Rutgers University, [2]Biology, 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. Furthermore, we recently found that the *D. melanogaster TART-A* telomeric transposon has captured a portion of the piRNA pathway 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 publicly available long-read genome assemblies from 107 *Drosophila*species. We identify 7 major telomeric retrotransposon clades from the *gag* gene and 6 major clades from the *pol* gene, including the HTT clade from the *melanogaster* subgroup and the TR2 clade found in the *ananassae* and *montium*subgroups. We find that telomeric TEs in two species of the *montium* subgroup captured a portion of the gene encoding a distinct piRNA effector protein, Piwi, and the telomeric transposons in multiple species of the *Zaprionus* genus captured portions of the PIWI-clade Argonaute protein Aubergine. The capture of an *aubergine* (*aub*) gene fragment appears to have occurred independently at least four times within the *vittiger* subgroup of *Zaprionus.* These gene capture events exhibit a complex pattern of gain, loss, and horizontal transfer among *Zaprionus* species. The repeated gain and loss of *aub* gene capture by telomeric TEs of *Zaprionus* is consistent with antagonistic evolution between these TEs and their host. We propose that gene capture by telomeric TEs represents a form of counter-silencing by TEs against their host.

316V **Pre-existing Mad binding site is required for novel expression pattern of *wingless* in *Drosophila guttifera* pupal wing** Takumi Karasawa[1], Namiho Saito[2], Shigeyuki Koshikawa[31]Division of Biosphere Science, Graduate School of Environmental Science, Hokkaido University, [2]School of Science, Hokkaido University, [3]Graduate School of Environmental Science, Hokkaido University / Faculty of Environmental Earth Science, Hokkaido University

Organisms have gained many kinds of traits and some traits are unique to one lineage. In evolutionary developmental biology, understanding the mechanisms underlying the gain of evolutionary novelty is one of the most important goals.

Drosophila guttifera has a novel pigmentation pattern on its wing. Its pigmentation spots are located on longitudinal vein tips, intersecting points of longitudinal veins and crossveins, and campaniform sensilla. This pigmentation pattern is regulated by *wingless*, which is expressed at the center of pigmentations during the pupal stage and promotes the expression of pigmentation genes such as *yellow*. In *Drosophila melanogaster*, on the other hand, *wingless* is expressed only on the crossveins and does not induce pigmentation (Werner *et al.* 2010). In a subsequent study, a *cis*-regulatory region that regulates *wingless* expression at vein tips and crossveins (gutCVT-core) was identified (Koshikawa *et al.* 2015). The orthologous region from *D. melanogaster* (melCV-core) drives *wingless* only on crossveins. Thus, the evolution in the *cis*-regulatory region brought the novel expression of *wingless* at vein tips and the novel pigmentations.

In this research, we narrowed down the required sequence for *cis*-regulation of *wingless* in gutCVT-core and compared its function with melCV-core.

First, we divided gutCVT-core into five parts. We then observed the regulatory function of gutCVT-core(s) that lack one or some of the parts. We found that two parts are essential for promoting expression at whole veins and one part is necessary for repressing expression at extra parts of longitudinal veins.

Second, we replaced some parts of gutCVT-core with homologous parts of melCV-core and observed the regulatory function of chimeric sequences. As a result, we found that the repressing function of gutCVT-core is also possessed by melCV-core.

Third, we knocked out the putative binding sites of Mad, which is a candidate for the upstream factor, and found that one of the binding sites is essential for promoting expression. Interestingly, melCV-core also had the homologous binding site, and knocking out the Mad binding sites in melCV-core caused the loss of expression at crossveins.

From these results, we concluded that a novel expression pattern of *wingless* by gutCVT-core is regulated through the combination of pre-existing sequences including the Mad binding site and newly gained sequences.

317V **Saltational episodes of reticulate evolution in the *Drosophila saltans* species group** Carolina Prediger[1,2], Samara Videira Zorzato[2], Erina A. Ferreira[1], David Ogereau[1], Aurélie Hua-Van[1], Lisa Klasson[3,3], Wolfgang Miller[4], Amir Yassin[1], Lilian Madi-Ravazzi[21]laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE), CNRS,IRD,Université Paris-Saclay, [2]São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences., [3]Molecular evolution, Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, [4]Center for Anatomy and Cell Biology, Department of Cell and Developmental Biology, Medical University of Vienna

Clarity in phylogenetic relationships is a requirement for many evolutionary studies, with bifurcating trees being the most resolved topology. However, competing topologies are obtained in many cases, either due to low taxonomic and character sampling (soft polytomy), or to complex speciation history (hard polytomy). We tested the effect of adding whole-genome data to resolve phylogenetic relationships between 15 species of the *Drosophila saltans* group, a clade of 23 Neotropical species classified in five subgroups. We conducted multiple approaches that progressively added data, namely, mitogenomics, subsampling genes from each chromosomal arm, concatenating whole genome codons with or without partitioning, and multilocus analysis of conserved syntenic blocks. The different approaches identified four time points on the tree with conflicting results: 1) at the origin of the *saltans* group, 2) at the split between the *saltans* subgroup and the *elliptica* and *cordata* subgroups, 3) at the origin of the *saltans* subgroup and 4) considering the relationships of the widespread species *D. prosaltans* with other species of the *saltans* subgroup. Tests based on the distribution of shared derived alleles and absolute divergence estimates identified distinct mechanisms potentially underlying those incongruences at the unresolved nodes, including rapid diversification, convergence in codon usage bias, hybridization, cytoplasmic introgression and incomplete lineage sorting. On other parts of the tree, well-resolved relationships were recovered. Therefore, phylogenomic data were still able to draw a picture of the speciation history of the *saltans* species group, although a more complex one than previously thought.

318V **Variation in the susceptibility of Drosophila spp. to disease; octanoic Acid in Drosophila sechellia host fruit protects the fly from fungal infection** Matthew Nikzad, Liam O'Malley, Jonathan Wang, Raymond St. LegerEntomology, University of Maryland, College Park

Adaptation to novel host plant species is a major driver of insect diversification due to many factors that include allowing access to new food sources and changing the competitive dynamic among species. While the fruit fly Drosophila melanogaster is found worldwide, closely related Drosophila sechellia is only found in the Seychelles, as a specialist of the Morinda citrifolia (Morinda) fruit, which is reported to be toxic to herbivores because it contains 1.2% Octanoic Acid (OA). We found that compared to other Drosophila species, D. sechellia is unusually susceptible to the very abundant broad-spectrum insect-killing fungus Metarhizium anisopliae. We confirmed that OA is toxic to Drosophila spp., other than D. sechellia, and we also found that OA is highly toxic to multiple insect pathogenic fungi by preventing germination. D. sechellia fed a diet containing OA, even at levels much less than found in Morinda fruit, had greatly reduced susceptibility to fungi which suggests that specializing to Morinda may have provided an enemy-free space, reducing reliance, and therefore adaptive prioritization, on a strong immune response. Drosophila species have very divergent lifestyles and thus provide a versatile model system for understanding the mechanisms of host-plant-pathogen interactions at different scales and in environmental context.

319V **Identifying the determinants of transposition during hybrid dysgenesis using pooled nanopore sequencing** Stefan Cerbin[1], Eva T Morison[2], Sophie Mosher[1], Justin Blumenstiel[11]Ecology and Evolutionary Biology, University of Kansas, [2]University of Kansas

Transposable elements (TEs) are endogenous mutagens that can excise themselves, cause double-stranded breaks, and trigger genome instability. The genome can control TEs in a myriad of ways and in Drosophila TE silencing is maintained by maternally provisioned piRNA. In syndromes of hybrid dysgenesis, transposons have been shown to activate in crosses where novel transposons in the paternal lineage are introduced into a female lacking these transposons and their respective piRNA. TE mobilization is proposed to be driven by the absence of piRNA provisioned in the maternal egg cytoplasm. However, we lack a complete understanding of the determinants of transposition during hybridization. For example, it has been proposed that the activation of some TE families can trigger the activation of others. In our study, we used a syndrome of hybrid dysgenesis in *D. virilis* to investigate genome-wide patterns of transposition. From dysgenic and non-dysgenic crosses, we backcrossed the F1 progeny and pooled ~500 flies for sequencing using Oxford Nanopore MinION. Using the resulting reads, we built a bioinformatic pipeline identifying novel insertions on a genome-wide scale. These data will allow us to build a model estimating the parameters driving transposition. This pipeline will allow us to predict, test, and identify transposon mobilization in *D. virilis*, unlocking some of the key determinants of large-scale genomic change in dysgenesis.

320V **The *Hairy E(spl)* gene complex of Holometabola and its dramatic remodeling in the evolutionary origination of brachyceran flies** Albert Erives, Guerin BrownBiology, University of Iowa

The *Drosophila* neurogenic genes encode the Notch-Delta signaling pathway and its target genes from the *Enhancer of split* and *Bearded* complexes, *E(spl)-C* and *Brd-C*. The complexes encode Hairy E(spl) (HES)-type basic helix-loop-helix

(HLH) transcription factors and Bearded Family member (BFM) proteins. Many *E(spl)-C* genes possess promoter-proximal enhancers containing the Su(H) paired sites (SPS) motif of inverted binding sites separated by 15 bp. We summarize findings from comparative analyses of the homologous complex of Coleoptera and other insect orders: **(1)** We show that the HLH and BFM genes of *E(spl)-C* and *Brd-C*, and the unlinked gene *HES-related* (*Hesr*) of *Drosophila* originated from an ancient holometabolan gene complex, which we refer to as *HES-C*. *HES-C* is composed of four SPS-driven genes in the order: *Hesr*<SPS, SPS>*Brd*, SPS>*mALPHA-BFM*, and SPS>*mBETA-HLH*. This configuration occurs in Hymenoptera, Coleoptera, Lepidoptera, and nematoceran lineages of Diptera, including neodipteran nematoceran lineages that are sister to Brachycera. **(2)** Using phylogenetic footprinting, maximal homology alignment, and targeted sequencing of beetle genomes, we show that *HES-C* is endowed with an array of conserved regulatory blocks, many of which contain unpaired Su(H) sites with specific satellite E-boxes, suggesting that Notch regulation is a pervasive feature of *HES-C*. Coleopteran *HES-C* has a total of 42 conserved regulatory blocks within the genomic region containing the four SPS regulatory blocks. A similar number of blocks is conserved in other insect orders, excepting Diptera, and many are homologous across Holometabola. **(3)** The BFM and HLH genes of *Drosophila* and other brachycerans originated from gene duplications that occurred in the stem brachyceran lineage. Moreover, in neodipteran nematocerans *HES-C* is maintained with a smaller array of conserved regulatory blocks than in other insect orders. This suggests that the gene duplications and splitting of *HES-C* into *Hesr*, *Brd-C*, and *E(spl)-C* in stem-brachycerans may have been evolutionarily preceded in stem-neodipterans by loss of individual enhancers from the ancient holometabolan *HES-C* regulatory array. These findings highlight the striking uniformity of regulatory architecture for *HES-C* across Holometabola and its dramatic remodeling that occurred in the evolutionary origin of Brachycera.

321V **Restoring the hidden micro-paralogical dimension to its rightful column in the study of gene regulatory evolution in *Drosophila*** John Reinitz[1], Albert Erives[21]Statistics, Ecology and Evolution, and Molecular Genetics & Cell Biology, University of Chicago, [2]Biology, University of Iowa

Maximal homology alignment is a new biologically-relevant approach to DNA sequence alignment that maps the internal dispersed microhomology of individual sequences onto two dimensions. It departs from the current method of gapped alignment, which uses a simplified binary state model of nucleotide position. In gapped alignment nucleotide positions have either no relationship (1-to-None) or else orthological relationship (1-to-1) with nucleotides in other sequences. Maximal homology alignment, however, allows additional states such as 1-to-Many and Many-to-Many, thus modeling both orthological and paralogical relationships, which together comprise the main homology types. Maximal homology alignment collects dispersed microparalogy into the same alignment columns on multiple rows, and thereby generates a two-dimensional representation of a single sequence. Sequence alignment then proceeds as the alignment of two-dimensional topological objects. The operations of producing and aligning two-dimensional auto-alignments motivate a need for tests of two-dimensional homological integrity. We present our work implementing basic principles for computationally testing the two dimensions of positional homology, which are inherent to biological sequences due to replication slippage and related errors. We then show that maximal homology alignment is more informative than gapped alignment in modeling the evolution of genetic sequences in *Drosophila*. In general, MHA is more suited when small insertions and deletions predominantly originate as local microparalogy. These results show that both conserved and non-conserved genomic sequences are enriched with a signature of replication slippage relative to their random permutations.

322V **IntroUNET: identifying introgressed alleles via semantic segmentation** Dylan D Ray, Daniel R SchriderUniversity of North Carolina

A growing body of evidence suggests that gene flow between closely related species is a widespread phenomenon. Alleles that introgress from one species into a close relative are often deleterious, but sometimes confer a significant fitness advantage. Numerous methods have therefore been devised to identify regions of the genome that have experienced introgression. Recently, supervised machine learning approaches have been shown to be highly effective for detecting introgression. One especially promising approach is to treat population genetic inference as an image classification problem, and feed an image representation of a population genetic alignment as input to a deep neural network that distinguishes among evolutionary models (i.e. introgression or no introgression). However, if we wish to investigate the full extent and fitness effects of introgression, merely identifying introgressed loci is not sufficient—ideally we would be able to infer precisely which individuals have introgressed material and at which positions in the genome. Here we adapt a deep learning algorithm for semantic segmentation, the task of correctly identifying the type of object to which each individual pixel in an image belongs, to the task of identifying introgressed alleles. Our trained neural network is thus able to infer, for each individual in a two-population alignment, which of those individual's alleles were

introgressed from the other population. We use simulated data to show that this approach is highly accurate, and that it can be readily extended to identify alleles that are introgressed from an unsampled "ghost" population, performing comparably to a supervised learning method tailored specifically to that task. Finally, we apply this method to data from *Drosophila*, showing that it is able to accurately recover introgressed haplotypes from real data. This analysis reveals that introgressed alleles are typically confined to lower frequencies within genic regions, suggestive of purifying selection, but are found at much higher frequencies in a region previously shown to be affected by adaptive introgression. Our method's success in recovering introgressed haplotypes in challenging real-world scenarios underscores the utility of deep learning approaches for making richer evolutionary inferences from genomic data.

323V **Functional and Evolutionary Analyses of Germline Stem Cell Regulating Genes across Select Drosophila and Outgroup Species** Luke Arnce, Jackie Bubnell, Charles AquadroCornell University

Germline stem cell (GSC) regulating genes are developmentally critical for reproduction in Drosophila. However, two GSC genes in D. melanogaster that are necessary for reproduction, bag of marbles (bam) and Female sterile (1) Yb (Yb), are not required for reproduction across closely related Drosophila species. It is unknown whether these examples of gene flexibility are exceptional for GSC function or a common phenomenon. The growing density of high quality full genome sequences within the Drosophila genus makes comparative analyses of GSC genes across Drosophila (and closely related outgroups) possible. We are characterizing the functional flexibility at GSC genes in Drosophila by further defining the functional evolutionary history of bam and broadly evaluating the conservation of GSC regulating genes.

324T **Low-level repressive histone marks fine-tune stemness gene transcription in neural stem cells** Arjun Rajan[1], Lucas anhezini[1], Noemi Rivas-Quinto[1], Megan C Neville[2], Elizabeth D Larson[3], Stephen F Goodwin[2], Melissa M Harrison[3], Cheng-Yu Lee[11]University of Michigan, [2]University of Oxford, [3]University of Wisconsin-Madison

Coordinated regulation of stemness gene activity by transcriptional and translational control poise stem cells for a timely cell-state transition during differentiation. Although important for all stemness-to-differentiation transitions, mechanistic investigation of the fine-tuning of stemness gene transcription *in vivo* is challenging due to the compensatory effect of translational control, a lack of sensitized functional readouts, and a lack of insight into the relevant transcription factors. We used the commitment to an intermediate neural progenitor (INP) identity to define the mechanisms that fine-tune stemness gene transcription in fly neural stem cells (neuroblasts). We demonstrate that the transcription factor Fruitless^{C} (Fru^{C}) binds regulatory modules of most genes uniquely transcribed in neuroblasts. Loss of *fru^{C}* function alone has no effect on INP commitment alone but drives progenitor dedifferentiation when translational control is reduced. Fru^{C} negatively regulates gene expression by promoting low-level enrichment of the repressive histone mark H3K27me3 in gene regulatory modules. Identical to *fru^{C}* loss-of-function, reducing Polycomb Repressive Complex 2 (PRC2) activity increases stemness gene activity. Studies in vertebrate stem cell lineages speculate that the occupancy of PRC2 subunits and the repressive histone mark H3K27me3 in regulatory modules of active genes contribute to the fine-tuning of stemness gene transcription. We propose low-level H3K27me3 enrichment fine-tunes stemness gene transcription in stem cells, a mechanism likely conserved from flies to humans.

325T **Pvr regulates cyst stem cell division in the Drosophila testis niche and has functions distinct from Egfr** Judy Leatherman, Nastaran MuesBiological Sciences, University of Northern Colorado

The rate of stem cell division is one of the key determinants of the abundance of differentiating progeny in stem cell-supported tissues, and mis-regulation can lead to tumorigenesis. The *Drosophila* testis stem cell niche is an excellent model system to study how stem cell division rates are regulated *in vivo*. This niche has two stem cell populations—the germline stem cells (GSCs) and cyst stem cells (CySCs), both of which cluster around a group of nondividing somatic cells called the hub. The differentiating cells of these two stem cell populations cooperate together to produce sperm. The epidermal growth factor receptor (Egfr) elicits signaling that is a key regulatory pathway in the cyst lineage, and much of the study of the CySC population has centered around understanding the complexities of roles played by Egfr. In this study we investigated the role of another receptor tyrosine kinase, *Pvr*, which is the sole *Drosophila* PDGF/VEGF homolog. We found that Pvr accumulates in the cyst lineage cells of the testis, while its ligand Pvf1 accumulates in the hub. *Pvr* inhibition in the cyst lineage cells by RNAi or dominant negative expression led to reduction in both CySC numbers and the proportion of CySCs in S phase, a result similar to that observed with Egfr inhibition. However, while Egfr inhibition led to a dramatic germ cell differentiation defect, Pvr inhibition caused only a low-penetrance germ cell phenotype in which spermatogonia failed to transition to spermatocyte differentiation. Overactivation by expression of constitutively active receptors revealed that both receptors failed to support germ cell differentiation. However, cyst

cells with constitutively activated Pvr promoted tumorous accumulation of cyst cells outside of the niche, a phenotype not observed with constitutively activated Egfr. These Thus, Egfr and Pvr have some shared functions and some receptor-specific functions in the cyst lineage of the testis.

326T **Loss of Piezo compromises embryonic post-wounding epidermal barrier function and survival** Alessandro Scopelliti, Luigi Zechini, Clelia Amato, Will WoodUniversity of Edinburgh (UK)

Tissue damage can occur in any part of our body following disease, injury, or chemical exposure. The consequent physiological self-healing and regenerative response is a highly controlled process leading to tissue repair and its failure can lead to serious and long-lasting consequences. Therefore, wounds need to be repaired quickly to prevent loss of tissue integrity and microorganisms colonization.

Using laser-induced wound on the embryonic ventral epidermis we showed that epidermal loss of the mechano-sensitive cation channel Piezo induces an acceleration of wound closure by affecting the acto-myosin cable dynamics at the wound edge.

Despite this initial beneficial effect, epidermal loss of Piezo has detrimental long-term consequences on tissue repair. After the initial closure, Piezo$^{-/-}$ epidermis deteriorates into a large gap at the wound site that fails to resolve and is accompanied by a persistent and progressively growing melanotic plug.

In addition, while control larvae developed from wounded embryos appear normal with no evidence of the preexisting wound, a large proportion of wounded Piezo$^{-/-}$ embryos develops into larvae exhibiting epithelial discontinuity, a prominent melanotic plug, and failure to assemble the cuticle layer in correspondence to the site of wound.

These persistent scars are permanent point of weakness that compromise the cuticle and epithelial barrier function ultimately resulting in high rate of mortality.

We propose a model whereby Piezo acts as molecular brake during wound healing, slowing down closure to ensure proper activation of repair mechanisms and re-establishment of a fully functional epithelial barrier.

327T **Transferrin 2 in homeostasis and ageing of the *Drosophila melanogaster* midgut** Anona Galbraith, David DoupéBiosciences, Durham University

Stem cells are responsible for maintaining tissue homeostasis. Loss of the balance between cell death and production, which often occurs with old age, can lead to a variety of diseases. The intestine is a tissue with a high turnover rate due to mechanical and chemical damage from digestion, making homeostatic control especially important. Previous work from our lab and others has shown that stem cell-secreted proteins play important roles in the control of *Drosophila* intestinal homeostasis. A screen of conserved secreted proteins expressed in intestinal stem cells identified the septate junction protein Transferrin 2 (Tsf2) as a regulator of homeostasis. We have found that changing the levels of Tsf2 expression in stem and progenitor cells in the *Drosophila* midgut disrupts homeostasis, and that Tsf2 expression changes with age. Furthermore, manipulating Tsf2 expression levels alters lifespan without impacting barrier function. Our findings may have relevance to human health and disease as the mammalian homologue of Tsf2 has been associated with tumorigenesis.

328T **The roles of SPARC and PLOD in *Drosophila* intestinal stem cell homeostasis** Paula Ferraces Riegas, David DoupéBiosciences, Durham University

Epithelia are constantly turned over as cells are lost from the surface and replaced by the proliferation of stem cells. Epithelial stem cells must be tightly regulated to maintain homeostasis and prevent over-proliferation. The intestinal stem cells of the *Drosophila* midgut are an ideal model system to identify regulators of intestinal stem fate, and study their function and regulation. We have identified PLOD and SPARC, regulators of collagen IV secretion and extracellular distribution respectively, as candidate regulators of the *Drosophila* intestinal stem cells. We are using RNAi knockdown and overexpression to explore their effects on intestinal stem cell proliferation and homeostasis. Initial results suggest that changes in SPARC and PLOD expression affect intestinal stem cell regulation and tissue structure in the *Drosophila* intestine. Both SPARC and PLOD are highly conserved in metazoans, raising the possibility of a conserved role in regulating intestinal stem cells.

329T **Stress-induced reversible cell cycle arrest requires PRC2/PRC1-mediated control of mitophagy in**

Drosophila germline stem cells and human iPSC Tung Chin Cheryl Chan, Julien C Ishibashi, Tommy C Taslim, Abdiasis C Hussein, Daniel C Brewer, Shuozhi Aaron Liu, Stuart C Harper, Bich N Nguyen, Jimmy C Dang, Debra Del Castillo, Julie C Mathieu, Hannele Ruohola=Baker, Riya Keshri, Scott Lyons, Ben Garver, Nanditaa Balachander, Samriddhi JhajhariaBiochemistry, University of Washington

Upon acute genotoxic stress, both normal stem cells and cancer stem cells can avoid apoptosis by entering the protective quiescent/G_0 phase, and later re-enter the cell cycle to regenerate lost daughter cells. Understanding the mechanism of this reversible quiescence stage in normal stem cells sheds light on targeting drug-resistant cancer stem cells. We took a novel approach to fill the critical knowledge gap of the regulation mechanisms at play during the entry and exit of stem cell quiescence. Here, we show that mTORC1-regulated, epigenetic-controlled mitophagy is required for radiation insult-induced quiescence in Drosophila germline stem cells (GSCs) and human induced pluripotent stem cells (hiPSC). Upon GSC irradiation, mito-fission (Drp1) or mitophagy (Pink1 and Parkin) are essential for entry into quiescence, whereas mitochondrial biogenesis (PGC1α) or fusion (Mfn2) are crucial during exit from quiescence. PRC2 complex components (Jarid2), and PRC1 complex (Sce), are required for mitophagy and for GSCs to enter quiescence. Importantly, hiPSC also require mTOR inhibition-mediated, epigenetically-regulated mitophagy to enter quiescence. We reveal that cyclinE decorating the outer mitochondrial membrane is concomitantly degraded with mitophagy. Hence, we hypothesize that mitophagy induced reduction of cyclinE is required for entry to G_0 phase. Together, this alternative method of G1/S control may present new opportunities for therapeutic purposes.

330T **Dad Regulates Germline Stem Cell Differentiation** Razeen Shaikh, Gregory T. ReevesArtie McFerrin Department of Chemical Engineering, Texas A&M University

The *Drosophila* ovarian germline is a powerful model system to study the dynamics of cellular decision-making. The asymmetric division of Germline Stem Cells (GSCs) to form two daughter cells — a self-renewed GSC and a differentiated Cystoblast (CB) — is the first symmetry-breaking event in the germline. The GSCs reside in a niche and are maintained by a short-ranged Dpp (ortholog of BMP2/4 in *Drosophila*) signal, which represses the key differentiation factor *bam*. The Dpp signal diminishes one-cell diameter away in the daughter cells, so that *bam* is derepressed, allowing for differentiation into CBs. This differentiation mechanism operating over a ~10-micron distance is robust to the variability in the levels of Dpp that the GSCs experience. Two regulatory loops may contribute to this robustness: Dpp signaling stimulates the expression of *Dad* and repression of *fused*. Dad inhibits Dpp signal transduction by deactivating the receptors, forming a negative feedback loop in the GSCs. Fused, along with Smurf, ubiquitinates the signaling complex in the cytoplasm, forming a positive feedback loop in the CBs.

We hypothesize that Dad regulates the extent of Dpp signal transduction in the GSC to enable the expression of *fused*, and subsequently *bam*, in the CBs. The cell division cycle is over ~12h, and the two dividing cells remain connected until the late telophase. We developed a mechanistic mathematical model of multi-compartment GSC division as the mass transfer interface connecting the two cells shrinks during cytokinesis, to investigate the dynamic roles Dad and Fused play in determining cell fate. We found that Dad optimally controls the Dpp signal transduction to enable GSC survival and differentiation. In Dad null mutants, GSCs were more likely to divide symmetrically. Our simulations also show that Dad and Fused make the GSCs and CBs robust to noise, respectively. Moreover, Dad and Fused work in tandem to ensure the GSC division is robust throughout the division cycle.

331T **Enteroendocrine cells affect intestinal regeneration through a gut-associated tissue signalling** Andre Medina[1,2], Julia Cordero[2,31]School of Cancer Sciences, Cancer Research UK Beatson Institute, [2]School of Cancer Sciences, University of Glasgow, [3]Cancer Research UK Beatson Institute

The last few years have witnessed an increased interest in the gastrointestinal tract beyond stem cell research. This is especially true for enteroendocrine (EE) cells, a secretory lineage from intestinal stem cells (ISCs) that plays a key role in sensing local and external stimuli. Similar to vertebrates, *Drosophila* has a diverse EE cell population scattered along the intestinal epithelium and organized into subpopulations of cells expressing different neuroendocrine (NE) peptides. EE cells and NE peptides are known to act locally and systemically, regulating whole body physiology. Locally, gut-derived NE peptides such as Bursicon and Tachykinin, show active role in ISC homeostasis. On the other hand, depletion of EE cells has a strong impact on intestinal regeneration. However, the mechanisms involved in the gut regenerative capacity of EEs is vastly unexplored. Here, we used adult *Drosophila melanogaster* as an in vivo model organism to study EE cells response to intestinal damage via regulation and secretion of NE peptides and their impact in the regenerative role of ISCs. Through a NE peptide RNAi screening, we discovered a novel role for Dh31, an orthologue of the mammalian

Calcitonin gene-related peptide (CGRP). We show here that Dh31 participates in intestinal regeneration by promoting ISC proliferation. Dh31 is upregulated by pathogenic damage-induced ROS or following treatment with harmful chemicals. Similar to its mammalian counterpart, secreted Dh31 communicates with the intestinal vasculature, the terminal tracheal cells (TTCs). Through Dh31-R activation and cAMP signalling, Dh31 regulates the secretion of niche factors from TTCs, necessary to support the regenerative capacity of ISC upon intestinal damage.

332T **Ovarian stem cell niche ageing involves changes in alternative splicing and reveals a role for the splicing factor *Smu1* in niche activity** Dilamm Even-Ros[1], Judit Huertas-Romero[1], Ildefonso Cases[1], Miriam Marín-Menguiano[1], Manuel Irimia[2], Federico Zurita[3], Acaimo González-Reyes[11]CABD, Spanish National Research Council (CSIC), [2]Centre for Genomic Regulation, Barcelona Institute of Science and Technology (BIST), [3]Genetics, Univ. of Granada

In many organisms, the ability to maintain tissue homeostasis -a property intimately linked to the correct activity of tissue-specific stem cells- declines with age. This decline can be caused by a reduction in the number of stem cells and/or by an alteration of their behavior, which ultimately depends on changes taking place in the stem cells themselves or in their microenvironment or niche. In our laboratory, we study how ageing affects stem cell niches and how this influences stem cell activity.

In the *Drosophila* ovary, a well-characterised stem cell niche model, ageing is associated with a reduction in the number of Germline Stem Cells (GSCs). By comparing the transcriptomic profiles of young *vs* old niche cells, we have identified a discrete number of alternative splicing events that affect terminal filament cells, cap cells and escort cells, the three cell types present in the GSC niche. We will present characterization of the known splicing factor Smu1 during GSC niche ageing. We have generated loss of function mutations and have made use of RNA interference to show that lack of Smu1 in the niche leads to stem cell loss. Our work advances our understanding of how alternative splicing regulates ovarian niche ageing and how Smu1 controls GSC numbers.

333T **Role of Non-Inflammatory Chemokines in Shaping Drosophila Midgut Epithelial Polarity During Regeneration** Mohamed Mlih, Jason KarpacCell Biology and Genetics, Texas A&M

Epithelial plasticity is crucial for dynamic remodeling of structure, size, and function of tissues. The adult Drosophila intestine (midgut) is extremely malleable, and can remodel/regenerate in response to external or internal cues, making it a genetically tractable model to study epithelial plasticity. Midgut regeneration is achieved through activation of intestinal stem cells that divide and differentiate to replace dying enterocytes, functionally differentiated epithelial cells. This regeneration process is tightly controlled to maintain epithelial structure, both the integrity and polarity. While basal polarity of the midgut epithelium has been extensively studied, less is known about the mechanisms involved in apical polarity during regeneration. We recently identified a secreted protein and integrin ligand, Diedel1, that is necessary for basal polarity of Drosophila midgut epithelium and enterocyte survival. By exploring the function of a Diedel1-like paralogous gene (CG34329), we established that Diedel proteins belong to a CC-motif chemokine ligand family. Chemokines are small, secreted proteins that create a gradient and provide directional cues for cells. We described this gene, CG34329, as CC-motif chemokine ligand 3 (CCL3), and uncovered that it is involved in apical polarity of the midgut epithelium. Using *in vivo* transgenic reporter flies, we found that CCL3 is mainly expressed in midgut enterocytes. To better understand the role of CCL3 in midgut epithelial plasticity, we generated CCL3 mutant flies using CRISPR-Cas9, and coupled with CCL3 RNAi-mediated knock down specifically in enterocytes, we found that CCL3 loss-of-function induces basal accumulation of enterocyte-like cells during regeneration, creating a multilayered epithelium lacking apical polarity. Finally, utilizing CCL3 NanoTagged transgenic flies under the control of the endogenous CCL3 promoter ($CCL3^{P}$-$CCL3^{Nano}$), we uncovered that CCL3 protein is secreted and accumulates in the midgut lumen (apical side of epithelium) where it can bind and direct intestinal progenitor/stem cells in response to regenerative cues. Our results confirm the existence of a cc-motif chemokine ligand family in Drosophila and highlight a key role for non-inflammatory chemokines in maintaining epithelial apical polarity during regeneration.

334T **Chondroitin sulfate is required for organ morphogenesis, maintenance, and regeneration in *Drosophila*** Collin Knudsen, Woo-Seuk Koh, Hiroshi NakatoGenetics, Cell Biology, and Development, 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. A small fraction of *Chsy* null mutants survive to adulthood, allowing the investigation of adult organ structures. We found that *Chsy* mutants show a defect in the wing maturation process, the last step of wing development. In the ovary, we observed a gradual disruption of the basement membrane and gross organ shape as the animals aged, indicating that CS functions in maintaining structural ECM integrity. 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. 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. Taken together, CS plays critical roles in morphogenesis, maintenance, and regeneration in an array of organs.

335T **Calcium influx rapidly establishes the distinct spatial Annexin recruitment patterns at cell wounds** Mitsutoshi Nakamura, Susan M ParkhurstBasic Sciences Division, Fred Hutchinson Cancer Center

When single cells composing tissues and organs are subjected to damage caused by environmental/physiological stresses, they must immediately initiate repair processes to survive this damage and remain functional. Extracellular calcium influx is the earliest event observed following injury in different cell types. This influx is thought to initiate cell wound repair processes comprised of rapid membrane resealing, dynamic cytoskeletal changes to close the wound, and cell cortex remodeling to restore original cortex organization/functions. Interestingly, we recently found that the *Drosophila* model requires not only an extracellular source of calcium, but also an intracellular source for optimal wound repair. While the calcium response upon wounding is uniform, it is responsible for the subsequent recruitment (within 15 seconds) of three RhoGEFs (RhoGEF2, RhoGEF3, and Pebble) to wounds in discrete spatial localization patterns. We previously found that AnnexinB9 (AnxB9), a calcium and phospholipid binding protein, functions to stabilize actin between this uniform calcium influx and RhoGEF2 recruitment to wounds. Drosophila has two other Annexins: AnxB10 and AnxB11. Intriguingly, all three Anxs are recruited to the wounds in discrete localization patterns within three seconds post-wounding. We find that AnxB10 and AnxB11 are required for RhoGEF3 recruitment to wounds. Interestingly, we find that the rapid Anx recruitment to wounds depends on calcium dynamics, rather than phospholipid dynamics, as none of the Anxs are recruited to wounds in the absence of extracellular calcium. Surprisingly, the depletion of intracellular calcium disrupts only AnxB10 and AnxB11 recruitment patterns to wounds. Thus, in addition to initiating the cell wound repair processes, our results suggest that calcium influx from both extracellular and intracellular sources are needed to break the uniform symmetry of the response and establish the distinct protein recruitment patterns of Anxs. We are currently investigating the role of calcium and other early cell wound repair factors on Anxs spatiotemporal patterning dynamics.

336T **Transcriptional regulation of germline stem cell identity** Amelie Raz[1], Yukiko Yamashita[1,21]Whitehead Institute for Biomedical Research, [2]Massachusetts Institute of Technology

Drosophila melanogaster male germ cells are continuously generated in the adult through asymmetric division of germline stem cells (GSCs). These divisions are perpendicular to a closed niche, such that GSCs always remain in contact with the niche and differentiating daughter cells are displaced. Though these asymmetric divisions have been well-characterized in their morphologic context, there are, to date, no transcriptional markers described that are exclusive to the GSCs and are not present in the differentiating daughter cell. Using single-cell RNA sequencing, we have identified a cohort of genes with enriched expression in putative GSCs. Validation with fluorescent *in situ* hybridization (FISH) reveals that several of these genes encode for transcripts with higher specificity to GSCs than any previously characterized. Phenotypic analysis has indicated that several of these factors may play a role in determining and maintaining GSC identity. Furthermore, this gene cohort is enriched for genes necessary for both male and female germline biology, possibly suggesting a specific role for GSCs in a «sex-undifferentiated» state. These data indicate that GSC division may include polarized RNA transport and/or perdurance, and provide new insight into the unique GSC identity.

337T **Investigating the Hippo Pathway's Role in Neuroblast Asymmetric Cell Division** Niranjan Joshi, Sherzod Tokamov, KathyAnn Lee, Richard FehonMolecular Genetics and Cell Biology, University of Chicago

The Hippo signaling pathway functions to restrict tissue growth in animals. However, studies have shown that Hippo pathway components also have functions not directly related to growth control. In particular, past studies found that Hippo pathway inactivation leads to aberrant apical polarity and failure of asymmetric cell division in *Drosophila* neuroblasts, and conversely that disruption of apical-basal polarity also disrupts Hippo pathway function. Here, we

report that a key upstream activator of the Hippo pathway, Kibra, localizes to the apical pole of the neuroblast during mitosis, similarly to the well documented localization of the Par components aPKC/Par6/Par3. We also find that polarized Kibra localization is dependent on aPKC and its kinase activity. We plan to use this system to further investigate the functional relationships between Hippo signaling and polarity and asymmetric cell division.

338F **Understanding the role of Vestigial in *Drosophila* wing imaginal disc regeneration** Surabhi Sonam, Rachel Smith-Bolton, Keaton SchusterCell and Developmental Biology, University of Illinois Urbana Champaign

Regeneration is a dynamic process that enables species to restore the structural organization and functional capacity of damaged or lost tissue structures. Our lab studies the biological principles underlying regeneration using a genetic ablation model in *Drosophila* wing imaginal discs. A key transcription factor Vestigial (Vg) is considered a selector gene that is both necessary and sufficient for establishing wing identity and for wing pouch growth. A previous study has shown that while Vg protein is reduced early during regeneration, the expression is eventually re-established in later stages of regeneration. We investigated the activity of two major enhancers controlling *vestigial* expression, the boundary enhancer (*vgBE*) and the quadrant enhancer (*vgQE*), during regeneration. Our preliminary data indicate that reporter expression controlled by the *vgBE* is maintained, albeit at reduced levels, in regenerating discs. By contrast, reporter expression controlled by the *vgQE,* which is thought to be crucial for wing growth and sustaining 'feed forward' signaling, is completely lost in early stages of regeneration and not restored until around 72 hours after damage. We aim to understand how wing disc regenerative growth is achieved despite the striking loss of *vgQE* activity and Vg expression during regeneration.

339F **Increases in calcium after dendrite injury drive dendrite regeneration** Vinicius Duarte[1], Katherine Louise Thompson-Peer[21]University of California, Irvine, [2]Developmental and Cell Biology, University of California, Irvine

After dendrite injury, peripheral nervous system neurons are able to mount a reliable, reproducible process of dendrite regeneration. Across many different classes of sensory neuron cell types, neurons can regenerate dendrite branches that partially restore receptive function to the cell. How neurons are able to sense injury to their dendrites, in order to initiate this regenerative response, is not known. In order to determine whether calcium signaling could play a role in injury detection, we expressed GCaMP in the class I and class IV sensory da neurons of the Drosophila larvae, and examined both calcium levels and the amount of regeneration in response to injury. First, we observe a massive increase in somatic calcium levels immediately after dendrite injury, with differences in the calcium increases induced by axon versus dendrite injury depending in the cell type. Second, we determined that a defect in calcium entry may explain the mechanism of how one of the strongest known suppressors of dendrite regeneration is able to block regeneration. Third, by comparing the unknown mechanisms of now neurons respond to dendrite injury with the more-well-characterized response to axon injury, we have identified calcium sources and signaling mechanisms that are essential for initiating dendrite regeneration after injury. Together our data suggests that neurons may use calcium signaling to sense dendrite injury, in potentially different ways from how neurons sense axon injury, in order to initiate a dendrite regeneration response.

340F **Piezo regulates wound closure to ensure effective inflammation and maintenance of epithelial integrity** Luigi Zechini, Alessandro Scopelliti, Clelia Amato, Will WoodUniversity of Edinburgh

Effective wound healing requires the fine balance between two crucial processes: re-epithelialisation and inflammation.

We exploited the genetic tractability and live-imaging potential of *Drosophila,* to visualize wounded ventral epidermal cells behaviour and track wound by confocal live-imaging.

Here, we explored the role of Piezo, a mechanosensitive channel responsible for calcium influx and sensing of changes in tissue tension – key molecular and mechanical consequences of tissue damage respectively – during *in vivo* wound healing.

We found that Piezo regulates both re-epithelialisation and inflammatory response upon wounding.

We observed that in the absence of Piezo, the wounded epidermis responds with a faster healing kinetics compared to control embryos. This faster wound closure is associated with increased intercalation rate at wound edge and by higher degree of actomyosin discontinuity.

Despite lower resting calcium levels, Piezo$^{-/-}$ embryos show comparable levels of calcium influx around the site of

wounding but reach lower peak values compared to the control. This is associated with reduced production of ROS and consequent impairment of macrophages recruitment. A rapid wound closure in Piezo mutants also leads to larvae with large melanotic plugs in the areas of epithelial discontinuity and with reduced survival of flies.

Overall, we found that Piezo is a key regulator of both re-epithelialisation and inflammation after epidermal wound.

341F **Mitochondrial lipid metabolism regulates JAK-STAT signaling and stem cell maintenance in the *Drosophila* testis** Rafael Demarco[1], Leanne Jones[21]Biology, University of Louisville, [2]Anatomy and Medicine/Geriatrics, University of California, San Francisco

The capacity of stem cells to self-renew or differentiate has been attributed to distinct metabolic states. A genetic screen targeting regulators of mitochondrial dynamics revealed that mitochondrial fusion is required for male germline stem cell (GSC) maintenance in *Drosophila melanogaster*. Depletion of *Mitofusin* (*dMfn*) or *Optic atrophy 1* (*Opa1*) led to dysfunctional mitochondria, activation of Target of Rapamycin (TOR), and a dramatic accumulation of lipid droplets (LDs). Pharmacologic or genetic enhancement of lipid utilization by the mitochondria decreased LD accumulation, attenuated TOR activation and rescued GSC loss caused by inhibition of mitochondrial fusion. However, the mechanism(s) leading to GSC loss were unclear. TOR activation has been demonstrated to suppress JAK-STAT signaling by stabilizing the JAK-STAT inhibitor SOCS36E. As JAK-STAT signaling is critical for regulating stem cell self-renewal in the testis, we wanted to test the hypothesis that the increase in TOR activity in early germ cells would lead to SOCS36E stabilization, which in turn, could contribute to stem cell loss. Indeed, we found that SOCS36E levels were higher in early germ cells upon depletion of *dMfn* or *Opa1*. Subsequently, we show that activation of the JAK-STAT pathway, but not BMP signaling, is sufficient to rescue loss of GSCs as a result of the block in mitochondrial fusion. In addition, preliminary data suggest that LD accumulation acts in parallel to TOR/SOCS36E to promote GSC loss. Our findings highlight a critical role for mitochondrial metabolism and lipid homeostasis in GSC maintenance, providing a framework for investigating the impact of metabolic diseases on stem cell function and tissue homeostasis.

342F **Cross-regulatory interactions among downstream targets of the master regulator genes Escargot and Stat92E in *Drosophila melanogaster* intestinal stem cells** Cynthia Petrossian[1], Courtney Frazier[1], Ithan Cano[1], Elizabeth Matz[1], Noah Barkan[1], Mariano Loza-Coll[21]Biology, California State University Northridge, [2]Biology, California State University, Northridge

Multicellular organisms maintain organ homeostasis in great part due to the activity of adult stem cells (ASCs) that replace cells that were lost to damage, disease or normal tissue turnover. ASCs can divide asymmetrically, giving rise to a new copy of themselves (i.e. self-renewal) and a sister cell that commits to differentiation into a specific cell type. A healthy ASC needs to make several decisions throughout its lifetime: 1) to divide or not; 2) if it divides, to self-renew or differentiate; 3) if it differentiates, into which cell type. Over the years, *in vivo* research on several model organisms has demonstrated the existence of genetic master regulators that control several aspects of ASC biology by controlling the expression and/or activity of potentially hundreds of downstream targets. While experimental work has mainly focused on manipulating downstream effects on an individual basis, less is known about how downstream targets may cross-talk with one another. To address this outstanding question, we use intestinal stem cells (ISCs) in the posterior midgut of the fruit fly (*Drosophila melanogaster*). We focused on Foxo and Indy, two targets of two well-known master regulator genes of these cells: the Snail family transcription factor Escargot (Esg) and the signal transducer protein STAT. Previous work in our lab showed that Esg/STAT manipulations caused an upregulation in Foxo and Indy expression. Surprisingly, the independent overexpression of Indy and Foxo did not phenocopy the manipulations of the MR genes. This would suggest that MR targets carry out their roles through complex, nonlinear interactions. To explore this idea, we manipulated the expression of Foxo and Indy, alone or in combination, using an ISC-specific and inducible Gal4/UAS system to knock down or overexpress these targets in intestinal progenitor cells. We then dissected the posterior midguts and used immunofluorescence microscopy to quantify morphological changes in intestinal cells and determine if dual manipulations would have additive or complex, nonlinear effects. We also determined the physiological and systemic effects of our localized genetic manipulations on fly lifespan and intestinal function using an intestinal barrier integrity (Smurf) assay. Our findings will hopefully improve our understanding of the potential unexpected effects of combination therapies, and thus contribute significantly to the development of new therapies in the context of regenerative medicine.

343F **Using the *Drosophila melanogaster* ejaculatory duct as a model to study postmitotic tissue regeneration** Navyashree Amruthahalli Ramesh, Allison Box, Jaimian Church, Laura ButtittaMolecular Cellular and Developmental Biology, University of Michigan

Regeneration of lost or damaged cells in many tissues involves “perfect regeneration” where activation of stem or progenitor cells repopulates lost cell types. However, tissues that are postmitotic with few or no stem cells, such as the heart, liver, kidneys, cornea, bladder, and brain can also regain partial tissue function after damage by engaging postmitotic regeneration strategies. In these postmitotic tissues it is not clearly understood how the tissues restore function and mass after damage or injury without changes in cell number. Cells in these postmitotic tissues exit the mitotic cell cycle permanently during development but retain the ability to enter a variant cell cycle lacking mitosis called endocycling, resulting in large, polyploid cells that can restore partial tissue function and mass. The fruit fly, *Drosophila melanogaster,* has emerged as an excellent model organism to study regeneration, and we have established the *Drosophila* Ejaculatory Duct (ED), a secretory organ of the somatic male reproductive system, as a model for postmitotic regeneration. *Drosophila* ED plays an important role in secreting components of seminal fluid including enzymes, sex and antimicrobial peptides, and is the location where they mix with sperm and move to the Ejaculatory bulb for transfer during mating. The cells of the ED are postmitotic and we investigated that these cells are polyploid in adults and the ED lack known progenitors or stem cells. Surprisingly, we have recently discovered that the ED can regenerate after damage. We find that undamaged cells in areas of induced cell death increase their cell size and ploidy, suggesting a mechanism termed “compensatory cellular hypertrophy” is triggered for regeneration of lost tissue mass. By 7 days post injury the organ regenerates completely, returning to the normal tissue size prior to injury. We are currently testing whether this tissue engages known or novel mechanisms for compensatory cellular hypertrophy during regeneration. Thus, this work will provide insight of the unique mechanism that is involved in the processes during the regeneration of the postmitotic cells.

344F **Elucidating the Role of *Btk29A* during early regeneration in *Drosophila*** Snigdha Mathure, Matthew Contreras, Mabel Seto, Benjamin Wang, Rachel Smith-BoltonUniversity of Illinois at Urbana-Champaign

Organisms are prone to damage and injury and possess varying regenerative capabilities depending on their species and developmental stages of life. Regeneration is an elaborate process of regrowth of damaged tissue or organs. *Drosophila* larval wing imaginal discs are the precursor structures that form the adult fly wings. The wing imaginal discs can undergo regeneration upon damage and have a complex morphology, including structural components crucial for cell shape and epithelial integrity. We study tissue damage and regeneration using a genetic ablation system that induces apoptosis in the wing primordium. One of the earliest responses after tissue damage is wound closure. Wound closure encompasses several mechanisms, such as reestablishing tissue continuity, clearing cellular debris, maintaining apicobasal polarity, and regulating the cellular adhesion architecture. An interplay between the epithelial morphogenetic cues and regenerative response is essential for the tissue to sense damage and initiate regeneration. How these morphogenetic regulators identify, induce, and regulate tissue regeneration is poorly understood. Thus, our goal is to characterize novel genes and cellular rearrangement mechanisms essential for initiating tissue regeneration. To achieve this goal, our lab previously screened for actin regulators important during regeneration and identified the gene *Btk29A*, the *Drosophila* ortholog of the mammalian Bruton’s tyrosine kinase (Btk), as vital for regeneration. Our preliminary data demonstrate that mutant *Btk29A/+* discs regenerate poorly and strongly suggest that changes associated with regeneration in *Btk29A/+* mutants occur early in regeneration, before 24 hours after damage. Thus, elucidating the role of *Btk29A* during wound closure, epithelial morphogenesis and initiation of the regenerative response will help us better understand the molecular underpinnings of early tissue regeneration.

345F **The cytoskeletal mechanics that shape a stem cell niche** Bailey N Warder[1], Kara A Nelson[1], Justin Sui[1], Lauren Anllo[1,2], Stephen DiNardo[11]Cell and Developmental Biology, University of Pennsylvania, [2]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 niche morphology impacts function. I use the *Drosophila* male gonadal niche to study mechanisms of niche formation, combining genetic tractability with powerful live-imaging techniques pioneered in our lab. 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). **I therefore hypothesize that actomyosin contractility (AMC) shapes the niche, and makes it more efficient in guiding GSC behavior**. Through transgenic and pharmacological manipulations, I show that AMC is required for generating a smoothened niche-GSC boundary and therefore a proper niche shape. Additionally, I 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 I have evidence that GSC divisions are required for MyoII polarity and niche morphogenesis. I therefore suspect that proper MyoII polarity in the niche is induced by forces inherent to GSC divisions. **Our work suggests a unique feedback mechanism where stem cells shape the niche that guides their behavior**. NIH Funding: F31HD105342, T32 GM007229 (BW), F32 GM125123 (LA), R35 GM136270 (SD)

346F **Regeneration following necrosis requires both apoptotic and non-apoptotic caspase activity** Jacob W KlemmArizona State University

Following instances of damage, apoptotic cells act as crucial signaling centers that set the stage for regeneration to occur. Studies in *Drosophila* imaginal discs have extensively characterized the signals released by apoptotic cells and have established several signaling mechanisms by which these signals dictate the collective tissue outcome. However, much less is understood about how tissues respond to unregulated forms of death, like necrosis. Necrotic cell death is rapid and disordered and is characterized by the loss of cell membrane integrity and release of cellular contents into the environment. To study the tissue response to necrosis, we established a genetic ablation system to drive necrotic cell death in the *Drosophila* wing imaginal disc.

With this model, we have found that tissues regenerate from necrotic injuries by a unique mechanism unlike other types of tissue repair. Significant apoptosis is induced in response to necrosis that, unlike other damage-associated apoptosis, is observed at a distance from the wound instead of the wound edge and is not regulated by the JNK signaling pathway. This necrosis-induced apoptosis (NiA) does not appear to participate in established apoptotic signaling mechanisms, yet NiA cells are required for regeneration; preventing NiA cell formation results in a reduced capacity to regenerate.

We are currently focused on understanding the pathways involved in NiA cell formation and the precise mechanism by which NiA cells promote regeneration. To understand which genetic pathways regulate NiA cell formation, we have been damaging different tissue compartments with necrosis. Interestingly, developmental pathways in the hinge and wing margin may restrict NiA cell formation to specific areas of the pouch. Identifying these pathways may also provide mechanistic insight into how NiA cells promote regeneration, as recent data suggests that NiA cells may not actually be apoptotic. Reporters of caspase activity suggest that NiA cells may survive caspase activation and persist into later stages of regeneration, where they appear to increase in number and migrate towards the damaged tissue. Moreover, caspase-sensitive lineage tracing reveals only a subset of NiA cells appear morphologically apoptotic. As NiA cells represent a unique regenerative response to tissue necrosis, further characterization may provide insight towards treating necrotic wounds in a clinical setting.

347F **Anillin-dependent Septin function is required to stabilize the actomyosin ring during cell wound repair** Viktor Stjepic, Justin Hui, Mitsutoshi Nakamura, Susan ParkhurstFred Hutchinson Cancer Center

Cells are under constant assault from various physiological/environmental stressors such that they require a robust wound repair response to restore the damage inflicted on their cortex. A key step in repairing cell cortex ruptures is the formation and translocation of an actomyosin ring – needed to pull the cortical cytoskeleton and the overlying plasma membrane closed. The mechanism of how this actin ring links to and pulls the membrane closed as it translocates inward is not known. We are investigating the role of Septins, a family of conserved GTP-binding proteins that have both membrane and cytoskeleton associated functions, in cell wound repair. *Drosophila* has 5 Septins separated into three classes, Sep1 and Sep4 (SEPT6 class), Sep2 and Sep 5 (SEPT2 class), and Pnut (SEPT7 class). Septins usually work in hetero-oligomeric complexes that polymerize into filaments, as well as higher-order structures. We find that all five Septins are recruited to wounds, albeit with different spatiotemporal dynamics. Interestingly, these differences occur in a class-independent manner. Knocking down any of the Septins results in impaired wound repair, including weak actomyosin ring formation and slower contraction rates. Pnut knockdowns exhibit a somewhat more severe phenotype including delayed wound closure. Our results indicate that Septins are performing non-redundant functions. Interestingly, we find that Anillin, a Septin and actin interacting protein, is rapidly recruited to the actomyosin ring and is crucial for the recruitment of Pnut and Sep1 to wounds. Anillin knockdowns exhibit a disrupted wound repair phenotype similar to that observed in Pnut knockdowns. Anillin is still recruited to wounds in Pnut knockdowns, suggesting that Anillin functions upstream of Septins in the context of cell wound repair. To further investigate how Septins and Anillin regulate actin ring dynamics during cell wound repair, we purified individual Septin and Anillin proteins to discern their actin bundling capabilities. Surprisingly, we find that Pnut and Anillin can bundle actin without forming hetero-oligomeric complexes. This individual capability of Pnut and Anillin to bundle actin is consistent with their severe individual

knockdown phenotypes, further highlighting the role of individual Septins and Septin complexes in cell wound repair.

348F **Nuclear lamina proteins underlie the non-canonical mode of asymmetric mitosis employed by Drosophila female germline stem cells** Julianna M. Hernandez[1], Tingting Duan[2], Pamela K. Geyer[11]Department of Biochemistry and Molecular Biology, University of Iowa, [2]Department of Medicine, University of Pittsburgh

Sustained gametogenesis throughout adulthood requires that the germline stem cells (GSCs) divide asymmetrically to give rise to one daughter that retains a stem cell identity and a second daughter that differentiates. We uncovered that Drosophila female GSCs employ a non-canonical mode of asymmetric mitosis, wherein chromosome segregation occurs in the presence of a largely intact nuclear envelope (NE) and nuclear lamina (NL). Execution of this specialized mode of mitosis requires insertion of centrosomes into the NL at the onset of mitosis and their removal at the end. As such, NL proteins have novel mitotic requirements. Indeed, GSC survival depends upon the NL protein emerin, also known as Otefin. Loss of emerin leads to mitotic NL distortion, causing changes in the structure of spindle microtubules and defects in chromosome segregation. Additionally, enlarged, active centrosomes are retained in the NL at the end of mitosis. Here, we are investigating the link between centrosome dysfunction and GSC loss in *emerin* mutants. We are defining how centrosome retention alters asymmetric divisions and whether retention of pericentriolar material on interphase centrosomes contributes to GSC loss. Together, these studies will define the role of the NL in execution of asymmetric mitoses critical for GSC maintenance.

349F **Escargot controls somatic cell fate by attenuating EGFR signaling** Jordan Kryza[1,2,3], Rafael Demarco[3,4,5], Ryan Mollenaur[3], Tony D. Southall[6], Andrea H Brand[7], D. Leanne Jones[1,2,3,8,9,101]Molecular Biology Institute, University of California Los Angeles, [2]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California Los Angeles, [3]Department of Anatomy, Division of Geriatrics, University of California San Francisco, [4]Biology, University of Louisville, [5]Dept. of Molecular, Cell and Developmental Biology, University of California Los Angeles, [6]Department of Life Sciences, Imperial College London, [7]The Gurdon Institute and Department of Physiology, Development and Neuroscience, University of Cambridge, [8]Dept. of Molecular, Cell and Developmental Biology, University of California, Los Angeles, [9]Eli and Edythe Broad Center for Regeneration Medicine, University of California San Francisco, [10]Department of Medicine, Division of Geriatrics, University of California San Francisco

Adult stem cells are essential for tissue homeostasis and repair. Cell fate is tightly regulated by many intrinsic, genetic factors and extrinsic cues that are present in the stem cell niche. Stem cell niches are labile; they respond to stimuli to maintain homeostasis, but the molecular details on how this is achieved are incomplete. Here, we explore one mechanism used to regulate the fate of somatic support cells in the *Drosophila* testis to maintain tissue homeostasis.

The hub is an essential component of the stem cell niche in the *Drosophila* testis that secretes factors that regulate the behavior of germline stem cells (GSCs) and somatic stem cells, known as cyst stem cells (CySCs). Our lab reported that both CySCs and hub cells require the Snail family transcriptional regulator Escargot (Esg) to maintain their identity. Targeted depletion of *esg* from hub cells resulted in loss of hub cells, due to their conversion to CySCs and commitment to CySC lineage. Subsequently, GSC and CySC populations are lost. Similarly, depletion of *esg* from early somatic cells results in loss of CySCs and precocious differentiation into daughter cyst cells. Together, these observations indicate that Esg plays a central role in determining somatic cell fates; however, the mechanism(s) by which Esg works to influence somatic cell identity are unknown.

In the adult testis, Egfr activity is low in hub cells under homeostatic conditions. However, in CySCs and early cyst cells, Egfr is active and is an important regulator of differentiation. By contrast, we observe that Esg protein is significantly higher in hub cells than in cyst cells. Therefore, we wanted to test the hypothesis that Esg influences somatic cell fate by repressing the Egfr signaling cascade. Indeed, we observe that repressing Egfr signaling suppresses hub cell to CySC conversion (H>C), upon depletion of *esg*. Conversely, overexpression of *esg* in CySCs increases the number of early cyst cells and results in conversion of CySCs to hub cells (C>H). CySC>Hub cell conversion was also dependent on EGFR activity, as constitutive activation of EGFR signaling in CySCs prevented C>H conversion upon *esg* overexpression. Taken together, data support the hypothesis that Escargot acts upstream of Egfr to regulate somatic cell identity and maintain tissue homeostasis in the testis.

350F **A novel, context-dependent role for the enteroendocrine cell lineage in intestinal tumorigenesis.** Maria Quintero, Erdem BangiBiological Science, Florida State University

The Drosophila intestinal epithelium, much like the mammalian, is maintained by stem cells that produce different

cell lineages required for proper tissue homeostasis. Cross-talk between the different cell types is essential to ensure proper cell lineage distribution and cell function. In the presence of cancer-driving mutations, these processes are often disrupted and co-opted to support tumorigenesis, but the mechanisms by which individual cell types contribute to disease progression are not well understood. Here, we utilize a novel genetic platform, PromoterSwitch, that allows the targeting of genetic manipulations to a small subset of cells expressing a cell-type-specific promoter and their subsequent progeny. With the use of PromoterSwitch, we generate large, transformed clones derived from individual adult intestinal stem/progenitor cells. We report that genetic alterations representing common colorectal cancer genome landscapes drive disruptions in cell fate decisions, altering the relative abundance of some cell lineages, and resulting in the emergence of abnormal cell fates. We also identify a novel, context-dependent tumor-promoting role for the hormone-producing enteroendocrine (EE) cell lineage in intestinal tumorigenesis. The mechanisms by which EEs drive tumorigenesis vary depending on the specific combination of genetic alterations present in the transformed tissue, emphasizing genotype-dependent nuances in cell-cell interactions underlying tumorigenesis. These findings offer a possible mechanistic explanation for previous clinical observations that colon tumors with high numbers of EEs are more aggressive with a worse prognosis and uncover novel potential vulnerabilities that could be exploited for therapy.

351F **Exploring the phenotypic effects of Toll signaling pathways in tumor progression in Drosophila models of EGFR-driven GBM** Julia Gonzalez Varela, Renee D. ReadEmory University

The treatment options for Glioblastoma (GBM) are currently limited to resection and chemotherapy approaches, but resistance to chemotherapy is common in patients. There is an urgent need for further understanding of the underlying biology of tumorigenesis in order to develop new treatment options. Previous genomic work on GBMs has shown that the epidermal growth factor receptor (EGFR) is frequently amplified and constitutively active in GBM tumors. To discover new factors that contribute to progression of EGFR-driven GBMs, we used our Drosophila GBM model in which GBM-like glial neoplasia can be induced by glial-specific overexpression of constitutively active forms of EGFR and dp110, a subunit of Phosphoinositide 3-kinase (PI3K), using the UAS-Gal4 gene expression system. Using our Drosophila GBM model system, we performed a modifier screen in which we searched for genes and pathways that worsened or 'enhanced' glial neoplasia when specifically overexpressed or activated in neoplastic glia. We also performed a transcriptome analysis comparing wild-type larval brains to those expressing a repo > dEGFRλ;dp110CAAX genotype. From our screen and RNAseq data, we identified the Toll pathway, which in Drosophila activates an NF-KB signaling pathway. Furthermore, the Toll pathway normally functions in innate immune and injury responses in the brain as well as in development and cell proliferation. Using our Drosophila GBM model, we are studying how the Toll signaling pathway and its effectors, including NF- KB, contribute to enhanced growth of neoplastic glial cells. We will use genetic manipulation approaches such as RNAi constructs under the UAS-Gal4 system to manipulate expression of Toll components in glia, and then evaluate phenotypic changes through volumetric analysis, cell counting, confocal microscopy, and immunohistochemical staining.

352S **Regulation of Regeneration by Damage-Responsive Maturity-Silenced Enhancers** John Quinn[1], Robin Harris[21]Arizona State University, [2]SOLS, 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 Maturity-Silenced (DRMS) Enhancers are regeneration regulatory regions that recognize damage stimuli and respond by activating genes essential for regeneration. To study DRMS enhancer activity, our lab uses the wing imaginal disc, which is highly regenerative in early L3 but loses this ability by late L3. This loss of regenerative capacity has been correlated to DRMS enhancer activity being silenced with maturity as it reaches late L3. Currently, we do not know how DRMS enhancers become activated following damage, or how they are repressed with maturity. My work focuses on identifying the damage-induced signals that activate DRMS enhancers and factors responsible for maturity dependent silencing. 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 DRMS 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 it is differences in levels of JNK signaling that is required for enhancer activation. In effort to determine other damage-

induced factors involved, RNA-seq of damaged wing imaginal discs showed the ligands of the JAK/STAT pathways are highly upregulated. To test if the transcription factor for the JAK/STAT pathway is involved, a new DRMS transgenic reporter was created lacking the JAK/STAT transcription factor binding sites which reduced enhancer activation. 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 DRMS activation.

Overall, my research aims to identify the minimal components necessary for DRMS activation, which might be used to activate DRMS in non-regenerative tissue to permit regeneration. In addition to this work, I am also investigating the factors essential for the maturity-dependent silencing of DRMS enhancers that could also be manipulated to improve regenerative capacity of a tissue.

353S **Homothorax is enriched in the adult Drosophila testis hub and is essential for its maintenance** Margaret de Cuevas, Erika MatunisCell Biology, Johns Hopkins School of Medicine

In the adult Drosophila testis, germline stem cells (GSCs) and somatic cyst stem cells (CySCs) adhere to a small cluster of somatic hub cells, which produce 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, delaminate from the hub, and convert into functional CySCs (Hétié et al., 2014). Thus, the adult Drosophila testis hub is a great genetic model for understanding niche plasticity.

Although some of the signals that regulate hub cells have been identified, much remains unknown about this small but essential cluster of cells. Following from the Fly Cell Atlas study (Li et al., 2022), Raz et al. (manuscript in revision) created a single cell RNA-seq resource covering all of Drosophila spermatogenesis, starting from in-depth analysis of adult testis single nucleus RNA-seq (snRNA-seq) data. With over 44,000 nuclei and 6,000 cells analyzed, the data provide identification of rare cell types including hub cells. Starting from a list of the most highly upregulated genes in adult hub cells, we performed a small-scale RNAi-mediated knockdown screen and found that *homothorax (hth)* is essential for maintenance of adult hub cells. *hth* encodes a homeodomain transcription factor that binds to another homeodomain transcription factor, encoded by *extradenticle (exd)*, and allows its nuclear import. Hth and Exd are Hox cofactors that are essential for patterning of the Drosophila embryo but also have additional Hox-independent functions throughout development.

Upon knockdown of *hth* within hub cells, we have found that the hub is rapidly lost, followed soon after, as expected, by loss of all GSCs and CySCs. We are currently investigating the mechanism for this loss of hub cells (cell death or conversion to CySCs), potential downstream targets of *hth* in the hub, and the role of its partner *exd* in maintaining the adult hub.

354S **Rap1 coordinates cell-cell adhesion and cytoskeletal reorganization to drive collective cell migration during embryonic wound healing** Katheryn Rothenberg, Rodrigo Fernandez-GonzalezUniversity of Toronto

Coordinated cell movements contribute to tissue development and repair and to the spread of metastatic disease. We investigate collective cell migration during wound healing in the *Drosophila* embryonic epidermis. Upon wounding, the cells immediately adjacent to the wound become polarized: cell-cell adhesion molecules are internalized from the wound edge, and actin and the molecular motor non-muscle myosin II accumulate at the interface with the wounded cells, forming a supracellular cable around the wound that coordinates cell movements. The cable is thought to assemble from and anchor at former tricellular junctions (TCJs) along the wound edge, which are reinforced during wound closure through the accumulation of adherens junction components. However, the mechanisms that coordinate the adhesive and cytoskeletal rearrangements required for rapid wound closure are unclear. We found that reducing the activity of the small GTPase Rap1 using a dominant-negative form of the protein (Rap1DN) slowed wound repair by 45%. The slower wound closure was accompanied by defective actomyosin polarization to the wound edge and a loss of E-cadherin at TCJs. Embryos expressing a mutant form of the Rap1 effector Canoe/Afadin that cannot bind Rap1 displayed a defect in E-cadherin accumulation at TCJs similar to the effects of Rap1DN, but with normal myosin polarization. This suggests that Rap1 signals through Canoe to drive TCJ reinforcement, but not to assemble the supracellular cable. To understand how Rap1 may be affecting actomyosin cable assembly, we measured Rho1/RhoA activity in Rap1DN embryos and showed that Rho1 activity was reduced by 72% in Rap1DN embryos, which was accompanied by a 54% reduction in tension at the wound edge. We found that the RhoGEF Ephexin, which can be activated by Rap1, was necessary for myosin polarization to the wound edge and rapid wound repair, with no significant effects on E-cadherin redistribution. Our data

support a model in which Rap1 simultaneously drives actomyosin cable assembly via activation of Rho1 through Ephexin and reinforces adhesion at TCJs via Canoe to enable rapid migration to heal embryonic wounds.

355S **Function of Traffic jam in regulating Drosophila ovarian stem cell niche cell fates** Nia Kang, Dorothea GodtCell and Systems Biology, University of Toronto

In a Drosophila germarium, three somatic cell types make a niche for germline stem cells: terminal filament, cap, and escort cells. Previously, we showed that the large Maf transcription factor Traffic jam (Tj) is important for cap cell specification and presence of escort cells. Tj is weakly expressed in cap cells and strongly in escort cells. We asked (i) whether the differences in Tj expression between cap and escort cells relate to their fate and (ii) what might cause the differential expression of Tj. The Notch pathway seemed a good candidate for regulating Tj expression, directly or indirectly, as Notch is also necessary for cap cell formation and has been suggested to work in parallel or upstream of Tj. We hypothesized that active Notch lowers Tj expression in cap cells, thereby promoting the cap cell fate. We confirmed that a reduction of N or Dl causes a reduced number of cap cells, and showed that this is unlikely due to cell death as P35 expression did not rescue the number of cap cells. Interestingly, the remaining cap cells in *N* or *Dl* mutants underwent a change in the shape and size of their nucleus and expressed higher levels of Tj, making them resemble escort cells. This raised the question whether the increased expression of Tj might be responsible for the supposed transition from cap cell to escort cell fate in *N* pathway mutants. To determine whether elevated Tj expression affects cap cells, we overexpressed Tj in cap cells. This resulted in a decreased number of cap cells, larger cap cell nuclei, and a lower signal intensity of the cap cell marker Lamin C, similar to *N* mutants. Together, our results suggest that cap cells - although known to depend on Tj expression for their fate - require a relatively low Tj expression level to maintain their characteristics and that the low Tj expression in cap cells depends on the N pathway.

356S **Assessment of cellular and functional heterogeneity within the *Drosophila* testis stem cell niche** Jennifer Viveiros[1], Erika Matunis[21]Cell Biology, Johns Hopkins School of Medicine, [2]Cell Biolgoy, 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 perform lineage tracing of PS 11-arising cells into the adult testis and simultaneously analyze protein expression in populations of hub cells. Additionally, we are using clonal analysis tools to determine the requirement of hub anchoring and maintenance genes across hub cells. 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.

357S **The impact of DNA damage response and cell cycle on germline stem cell survival in the *Drosophila* testis** Jasmine Grey[1], Salman Hasan[2], Erika Matunis[11]Cell Biology, Johns Hopkins University School of Medicine, [2]Johns Hopkins University School of Medicine

Germline stem cells (GSCs) have the unique ability to transmit the genome to the next generation, making their DNA repair strategies of particular interest. The most consequential kind of DNA damage is the double stranded break (DSB). Several highly conserved repair pathways have evolved to sense and repair this otherwise cell-lethal event. The nonhomologous end joining (NHEJ) repair pathway predominates in the G1 phase of the cell cycle but is often mutagenic. In contrast, the homologous recombination (HR) repair pathway is non-mutagenic but is limited to the S and G2 phases of the cell cycle. Many stem cell types, including GSCs, are relatively resistant to DNA damage. Here, we use the well characterized *Drosophila* testis stem cell niche to investigate the response of stem cells to DNA damage caused by ɣ-irradiation (IR). Preliminary data from our lab found that 60% of GSCs are lost one day after exposure to 75 Gy of IR. Preliminary data also shows that DSBs must be repaired using the HR pathway and not the NHEJ pathway for GSCs to repopulate the niche one week after IR. Additionally, we have found that GSCs lost upon irradiation do not die in the

niche; they instead leave the niche and are later lost. Whether they are lost by death or differentiation is not yet known. I hypothesize that the GSCs receiving DNA damage during S or G2, which allows for access HR, are selectively retained in the niche. To test this, I will combine the *Drosophila*-specific fluorescent ubiquitination-based cell cycle indicator (fly-FUCCI) system and live imaging to examine the cell cycle phase of GSCs at the time of irradiation. I will follow this by determining whether the GSCs that leave the niche are lost to differentiation or cell death by fixing and staining the testes for cell death and differentiation markers after live imaging. This research will provide further insight into how adult stem cell populations can survive exogenous DNA damage and maintain the health of the tissue. A deeper understanding of stem cell resistance to exogenous DNA damage will have implications for other radio-resistant stem cells, like cancer stem cells, which contribute to the regrowth of the tumor after radiotherapy.

358S **DNA replication establishes asymmetric sister centromeres epigenetic** Rajesh Ranjan, Xin ChenBiology, 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-genic information could be inherited differentially to establish distinct cell fates is not well understood. Here, we report a series of spatiotemporally regulated asymmetric components, which ensure biased sister chromatid attachment and segregation during ACD of Drosophila male germline stem cells (GSCs). First, sister centromeres are differentially enriched with proteins involved in centromere specification and kinetochore function. Second, temporally asymmetric microtubule activities and polarized nuclear envelope breakdown allow for the preferential recognition and attachment of microtubules to asymmetric sister kinetochores and sister centromeres. Abolishment of either the asymmetric sister centromeres or the asymmetric microtubule activities results in randomized sister chromatid segregation. Together, these results provide the cellular basis for partitioning epigenetically distinct sister chromatids during stem cell ACDs.

However, how asymmetric sister centromeres form with identical genetic sequences is not well understood. I performed Super-Resolution Chromatin Fiber (SRCF) assay to visualize newly replicated sister chromatids. I found that one sister chromatid inherited more CENP-A compared to the other one, suggesting pre-existing (old) CENP-A is recycled asymmetrically during DNA replication. Further, the inner-kinetochore protein CENP-C shows a similar asymmetric pattern, the sister chromatid with higher CENP-A density also shows higher CENP-C density than the other sister chromatid, indicating a relay of asymmetry from centromere to kinetochore. I found that the CAL1 chaperone for CENP-A is required for both old CENP-A recycling during the S-phase and new CENP-A incorporation during the S/G2 phase in male GSCs. Compromising CAL1 activity abolishes both CENP-A and CENP-C asymmetry and results in mis-determination of GSC and progenitor germ cell fate, resulting in both GSC loss and early-stage germline tumor phenotype. Furthermore, compromising lagging strand polymerases, Pol α and Pol δ, significantly increases asymmetric sister-centromere formation in symmetrically dividing progenitor cells. We propose that the rate of lagging strand synthesis biases the pre-existing CENP-A recycling and hence helps establish asymmetric sister-centromeres. Together, these results show the molecular mechanisms underlying the establishment of asymmetric sister centromeres in GSCs and open new directions to study these phenomena in other biological contexts.

359S **Investigation of Follicle Stem Cell division rate regulation with FUCCI cell cycle reporters** David Melamed[1], Aaron Choi[1], Amy Reilein[1], Daniel Kalderon[21]Columbia University, [2]Biological Sciences, Columbia University

Drosophila ovarian Follicle Stem Cells (FSCs) present a favorable paradigm for understanding how stem cell division and differentiation are balanced in communities where those activities are independent. FSCs also allow exploration of how this balance is integrated with dynamic, spatial stem cell heterogeneity. FSCs are located near the mid-point of the germarium and are arranged principally in two adjacent rings lining the circumference of the germarium. The more posterior ring of about eight FSCs directly produces 5-6 proliferative Follicle Cells (FCs) in each 12h egg chamber budding cycle, while anterior FSCs can move further anterior to produce 1-2 quiescent Escort Cells (ECs) in the same period. A single stem cell lineage can produce both FCs and ECs over time because FSCs readily amplify and can move between anterior and posterior locations. Thus, the FSC community exhibits dynamic spatial heterogeneity. EdU incorporation has been used to approximate division rates, suggesting that posterior FSCs divide 60-70% faster than anterior FSCs. Here, by using live imaging and FUCCI cell-cycle reporters, we measured absolute division rates and found that posterior FSCs cycle almost four times faster than anterior FSCs, with considerably shorter G1, G2 and S phases. The measured division rates are consistent with the rate of production of FCs from posterior FSCs and therefore suggest that there is no net flow between anterior and posterior FSCs. Exchange in both directions was observed during live imaging with FUCCI markers and using H2B-RFP dilution to reflect division histories. Inversely graded Wnt and JAK-STAT pathway signals regulate

FSC differentiation to ECs and FCs. JAK-STAT signaling promotes FSC cycling as well as differentiation to FCs, affording some coordination of these activities. When JAK-STAT signaling was manipulated to be spatially uniform, the differential between posterior and anterior FSC division rates was reduced but remained substantial, showing that graded JAK-STAT signaling only partly explains the graded cycling of FSCs. By using FUCCI markers, we found evidence for a G2/M cycling restriction of wild-type FSCs in addition to a graded G1/S restriction, and that JAK-STAT signaling promotes both G1/S and G2/M transitions.

360S **Harnessing Drosophila for In Vivo Evaluation of Radioprotection Conferred through Consumption of Radiation-Resistant Yeasts** Robert Volpe, Rachel CoxUniformed Services University of the Health Sciences

Since the discovery of diverse fungi thriving in the highly irradiated structures surrounding the exploded reactor at the Chernobyl Nuclear Power Plant, fungi have risen to prominence as highly radiation-resistant organisms. Drosophila have recently emerged not only as a desirable model for studying intestinal stem cell health and the gut microbiome, but also an equally suitable model for studying radiation injury to the gut. As natural yeast-feeding organisms, Drosophila are well-suited to screen radiation-resistant yeast for radioprotective effects on radiosensitive intestinal stem cells. Here, we evaluate the radioprotective influence of dietary administration of diverse highly radiation-resistant yeasts to Drososophila irradiated with varying doses of gamma radiation on general health markers as well as their intestinal stem cell population.

361S **Melanization regulates wound healing by limiting polyploid cell growth in the *Drosophila* epithelium** Loiselle Gonzalez[1], Vicki Losick[2], Elizabeth Mortati[21]Biology, Boston College, [2]Boston College

Tissue damage is an inevitable risk for all organisms and results in cell loss and an open wound. As a consequence, organisms quickly repair their wounds through either cell division or cell growth by becoming polyploid. Polyploid cells contain more than the diploid copy of the genome and often arise in response to stress, including tissue injury. Previous studies have shown that wound healing in the *Drosophila* abdominal epithelium requires epithelial cells to both endocycle and fuse generating a giant multinucleated, polyploid cells that is required to restore tissue mass, integrity, mechanical forces, and protect against genotoxic stress. Yet, despite its essential role in wound healing the signals that initiate and regulate the extent of polyploidy remain poorly understood. Following a puncture wound, a melanized scab forms within hours at the site of injury via activation of phenol oxidases, encoded by prophenoloxidase genes (PPO1, PPO2, and PPO3). Melanization is known to both repair the damaged insect cuticle as well as mount immune response to microbial infection. In addition, we have observed that the epithelial polyploid cell arises at the site melanization and in direct contact with the scab. Using a triple PPO null mutant, we have now discovered that melanization is required for epithelial wound closure leading to an exacerbated growth response that extend well beyond the site of injury. Therefore, the extent of polyploid cell growth, both endocycle and fusion, during wound repair is dependent on melanization.

362S **Multinucleated, polyploid cells arise and protect against UV-A irradiation in the adult *Drosophila* epithelium** Minqi Shen[1], Benjamin Lamarre[2], Vicki Losick[21]Biology, Boston College, [2]Boston College

Many cell types, including cancer cells, use endocycle as a means to sustain growth in the presence of genotoxic stress by epigenetically silencing p53-dependent genes as well as targeting p53 for proteolytic degradation. However, polyploid cells can also arise by endomitosis and cell fusion leading to generation of multinucleated, polyploid cells or syncytia. Therefore, it remains poorly understood how ploidy state (mono- vs multinucleated) are stimulated by and protect against genotoxic stress. In *Drosophila*, we have found that wound healing and aging in the adult abdominal epithelium induces cell fusion and the generation of multinucleated, polyploid cells in the presence of DNA damage. Therefore, we hypothesized that UVA irradiation can also stimulate the generation of multinucleated cells, which may then serve to protect against genotoxic stress. To do so, adult *Drosophila* were irradiated with 25-50mJ of UV-A leading to p53-dependent cell loss over 5 days. By 7 days post UVA, we found that epithelial integrity was restored via generation of both mono- and multinucleated polyploid cells which appear to arise by cell fusion and the endocycle. However, the multinucleated cells were predominant and inhibition of cell fusion by expression of a dominant negative Rac GTPase revealed that cell fusion is essential for epithelial cell survival following UV-A irradiation. Therefore, we are working to elucidate the signals that regulate cell fusion in response to UV-A as well as how multinucleated, polyploid cells regulate the p53-DNA damage response to maintain tissue integrity under conditions of genotoxic stress.

363S **Real-time Notch signaling mechanisms driving intestinal repair** Hsuan-Te (Miriam) Sun, Erin Sanders, Andrew Labott, Javeria Idris, Saman Tabatabaee, Lucy O'BrienStanford University

The gastrointestinal epithelium is the major frontline defense against a constant barrage of environmental insults. Failure to appropriately respond to environmental insults results in loss of tissue integrity and ultimately death. To maintain intestinal tissue homeostasis, resident stem cells must appropriately tune their differentiation rates to the organ's needs. These processes are controlled by the Notch signaling pathway. Here, we use single-cell resolution live imaging of Notch signaling kinetics in the adult *Drosophila* midgut to reveal that real-time activation of Notch is accelerated nearly two-fold during injury, consistent with the organ's acute need for replacement cells. We also find that faster stem cell differentiation is accompanied by widespread perdurance of the Notch ligand Delta as well as nuclear exclusion of the Notch co-repressor Groucho; this suggests that suspension of canonical Delta-Notch lateral inhibition may underlie the accelerated kinetics of Notch activation seen during regenerative conditions. By understanding how changes in real-time Notch signaling direct stem cell fate programs, we will gain fundamental insights into the role of Notch in effecting whole-organ repair responses.

364S **Rab11 mediates E-cadherin recycling during embryonic wound healing** Sofia Karter Lopez, Kate MacQuarrie, Katheryn E. Rothenberg, Rodrigo Fernandez-GonzalezUniversity of Toronto

Collective cell movements drive the formation and repair of tissues in development and the spread of metastatic disease. To understand how cells coordinate their migration, we investigate wound healing in the epidermis of Drosophila embryos. Upon wounding, a supracellular cable composed of the cytoskeletal protein actin and the molecular motor non-muscle myosin II assembles around the wound, creating tension to coordinate cell movements and drive wound closure. The actomyosin cable forms through the polarization of actin and myosin in the cells adjacent to the wound. In parallel, adherens junction proteins, including E-cadherin, are depleted from former bicellular junctions 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 the former bicellular junctions via endocytosis, but it is unclear how E-cadherin is delivered to TCJs, and whether the E-cadherin accumulating at TCJs is recycled protein that was previously endocytosed or protein originating elsewhere in the cells. To examine the potential role of protein recycling in E-cadherin accumulation at TCJs, 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 TCJ decreased by 33%. Together, our results suggest that Rab11 is partially responsible for E-cadherin remodeling during wound repair, and that cell-cell adhesion rearrangements control the rate of wound healing independent of cytoskeletal polarity. Future work will investigate the role that other endocytic and recycling compartments, as well as diffusion of junctional material along cell membranes play in the redistribution of E-cadherin and rapid wound repair.

365V **Kinetics of blood cell differentiation during hematopoiesis revealed by quantitative long-term live imaging** Kevin Ho[1], Rosalyn Carr[2,3], Alexandra Dvoskin[1], Guy Tanentzapf[11]Department of Cellular and Physiological Sciences, University of British Columbia, [2]School of Biomedical Engineering, University of British Columbia, [3]British Columbia Children's Hospital

Stem cells typically reside in a specialized physical and biochemical environment that facilitates regulation of their behavior. For this reason, stem cells are ideally studied in contexts that maintain this precisely constructed microenvironment while still allowing for live imaging. Here, we describe a long-term multi-organ co-culture and imaging strategy for hematopoiesis in Drosophila that allows tracking of multiple blood cell behaviors for 14-16 hours. We find that fly blood progenitors undergo symmetric cell divisions and that their division is both linked to cell size and is spatially oriented. Using quantitative imaging to simultaneously track markers for stemness and differentiation in progenitors, we identify two types of differentiation that exhibit distinct kinetics during transition of blood cell identity. Following infection, we observe a shift between the two types of differentiation and a change in their spatial distribution and differentiation speed, showing that infection-induced activation of hematopoiesis occurs through modulation of the kinetics of cell differentiation. Overall, our results show that even subtle shifts in proliferation and differentiation kinetics can have large and aggregate effects to transform blood progenitors from a quiescent to an activated state.

366V **Wdr4 Limits Intestinal Stem Cell Division and has conserved function for Gut Homeostasis** Kreeti Kajal[1], Elham Rastegari[2], Pei-Rong Lin[3], Ruey-Hwa Chen[3], Wendy W Hwang-Verslues[4], Hwei-Jan Hsu[11]Molecular and Biological Agricultural Sciences Program, Taiwan International Graduate Program Institute of Cellular and Organismic Biology, National Chung Hsing University and Academia Sinica, Taipei 11529, [2]Department of Integrative Biology and Pharmacology McGovern Medical School at the University of Texas Health Sciences Center, Houston, United States,

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WD40 repeat protein, Wdr4, is known to control the differentiation of embryonic and germline stem cells (GSCs) via protein-protein interactions. However, the physiological significance of Wdr4 in animal health and somatic stem cells remain unclear. Here, we report that Wdr4 loss in *Drosophila* causes shortened lifespan, locomotor defects, and increased susceptibility to stress. Wdr4 depletion also results in aberrant midgut morphology and dramatically increased proliferation of intestinal stem cells (ISCs) toward symmetric divisions, at least in part via JNK signaling activation, leading to increased ISCs accompanied by decreased absorptive enterocytes (ECs). To maintain gut integrity, the remaining ECs increase cell size by extra rounds of endoreplication. In addition, ECs display disrupted cell-cell contact, leading to leaky gut, increased immune response probably due to infection, and redox stress, which is known to non-cell autonomously promote ISC proliferation. Extensive clonal analysis showed that the Wdr4 depleted ISCs has increased proliferative capacity and JNK signaling. Interestingly, overexpressing human WDR4 partially rescues *wdr4* mutant gut phenotypes, and mouse small intestine carrying *wdr4*-depleted $Lgr5^{+}$ ISCs exhibit more mitotic cells, indicating a conserved role of Wdr4 in ISCs from insects to mammals. Currently, we are trying to investigate the Wdr4 interacting partner, which regulates ISC function. These results document the complexity of ISC regulation by intrinsic and local signals, and may shed the conserved function of Wdr4 for somatic stem cell function.

367V **Role of Acetyl-CoA Carboxylase in regulating Drosophila Ovarian Germline** Oyundari Amartuvshin, Yi-Ting Ke, Hwei-Jan HsuAcademia Sinica

Stem cell asymmetric division, self-renewal and differentiation is critical for tissue homeostasis. Through our RNAi-based screening to identify metabolic enzymes that control oogenesis, we spotted Acetyl-CoA Carboxylase (ACC) as one of our candidates. ACC catalyzes carboxylation of acetyl-CoA to form malonyl-CoA, a rate-limiting step for de novo lipogenesis. Here, we report that ACC controls endosomal trafficking in the Drosophila germline for functional reproduction. In addition, ACC depletion shows mitotic abnormalities such as centrosome overduplication, spindle mis-orientation and polyploidy. Finally, ACC depletion leads to in creditably increased acetylated tubulin and cortactin levels, and decreased lipid synthesis. We are now investigating if Acetyl-CoA accumulation or lack of fatty acids account for the observed phenotypes.

368V **Investigating the Role of Asperous in Tissue Regeneration** Si CaveMolecular Sciences, Arizona State University

The mechanisms of tissue regeneration are not fully understood. Using the model of the *Drosophila* wing imaginal disc, we have identified factors upregulated during the regenerative process through RNA-sequencing. Asperous (CG9572) is an uncharacterized extracellular protein that is one of the most upregulated genes during regeneration. Utilizing computational tools to predict protein structure, we found the presence of a WD40 domain, suggesting a role in coordinating multi-protein complexes. Additionally, a BLAST search has shown Asperous has many similarities to the members of the Nimrod protein family, which are involved in the phagocytosis of apoptotic bodies through complex assemblies.

To characterize the role of Asperous during regeneration, we used our novel genetic ablation system to induce cell death via apoptosis or necrosis while simultaneously manipulating the surrounding tissue. We have found that Asperous is essential for regeneration when cell death is induced via apoptosis, but it not essential for regeneration when cell death in induced via necrosis. Moreover, when Asperous is knockdown in surrounding tissue of necrotic-induced damage, it improves regeneration. Given that Asperous may be contributing to the clearance of apoptotic bodies, we have begun to characterize this in both regenerative contexts. The dual role of Asperous in different tissue regeneration backgrounds, makes it an important target for further investigate.

369T **The synaptonemal complex plays multiple roles in establishing the recombination landscape across chromosomes** Katie Billmyre[1], R. Blake Billmyre[2], R. Scott Hawley[21]Stowers Inst Med Res, [2]Stowers Institute for Medical Research

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 hypothesize this behavior is explained by a difference in the timing of meiotic events on individual chromosomes or the importance of chromosome structure as the X and autosomes have different structures. To distinguish between these possibilities, we are currently using a two-pronged approach of super-resolution imaging and genomics to examine the relationship between the SC, double-strand breaks, and recombination on the X chromosome and the autosomes. Our sequencing results show 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.

370T **Role of PIP2 in sperm head formation** Marynelle S Icmat[1,2], Negar Nasirzadeh[1,3], Julie A Brill[1,21]Molecular Genetics, University of Toronto, [2]Cell Biology, The Hospital for Sick Children, [3]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. It starts with asymmetrical division of a germline stem cell to form a new stem cell and a daughter cell for differentiation. The committed daughter cell will undergo mitosis, meiosis and an elaborate pathway of cellular differentiation that will determine the final shape of the sperm. These processes are collectively known as spermatogenesis and are highly conserved from mammals to flies, yet we lack knowledge as to how these processes are regulated. An important phenomenon during spermatogenesis is the attachment of the sperm tail to the head, a process known as sperm head-tail coupling. In *Drosophila melanogaster*, 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. Defects in this process result in abnormal sperm head formation and lead to male infertility. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) which is synthesized by the PIP5-kinase Skittles (Sktl) in Drosophila, plays a role in proper basal body docking. As null mutations in Sktl result in lethality, our lab developed a tool to deplete PIP2 by expressing the Salmonella phosphatase SigD using a Drosophila testis-specific promoter. SigD expression results in depletion of PIP2 and male sterility. In PIP2-depleted spermatids, 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 co-expression of Sktl, indicating essential roles of Sktl and PIP2. Understanding the mechanisms through which Sktl and PIP2 regulate sperm head formation will aid in uncovering novel insights into the conserved processes that regulate sperm development.

371T **Eukaryotic initiation factor 4E-5 is essential for spermatogenesis in *Drosophila melanogaster*** Brook L Falk[1,2], Lisa Shao[1,2], Jaclyn Fingerhut[3], Hong Han[4], Elizabeth Hall[1], Vincent Lee[1], Giovanna Maldonado[5], Yeumeng Qiao[1,2], Liang Chen[4], Gordon Polevoy[1], Greco Hernández[5], Paul Lasko[4], Julie Brill[11]The Hospital for Sick Children, [2]University of Toronto, [3]Whitehead Institute for Biomedical Research, [4]McGill University, [5]National Institute of Cancer

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 paralogs of eIF4E, including four testis-enriched paralogs (eIF4E-3, -4, -5 and -7). Other than eIF4E-3, none of these has been characterized genetically. We used CRISPR/Cas9 mutagenesis to generate mutants of *eIF4E-5* and discovered that it is essential for male fertility. *eIF4E-5* mutants exhibit defects during post-meiotic stages, including fully penetrant defects in individualization resulting in failure to produce mature sperm. These defects can be rescued through expression of a 3xFLAG tagged *eIF4E-5* transgene, which also revealed that eIF4E-5 localizes to the distal ends of elongated spermatids where many mRNAs are known to localize. During individualization, organelles and cytoplasmic components not needed in mature sperm are stripped away by non-apoptotic caspase activity regulated by accumulation of the cullin-3 E3 ubiquitin ligase complex inhibitor Soti at the distal ends of the sperm tails. *soti* transcripts localize to the distal ends of spermatid tails and *soti* mutants exhibit similar defects during individualization to what we observe in *eIF4E-5* mutants, leading us to hypothesize that *soti* is a target of eIF4E-5. Overall, our results indicate that eIF4E-5 regulates translation in a spatiotemporal manner to control processes important for spermiogenesis and male fertility.

372T **Prostaglandins limit nuclear actin to control nucleolar function during oogenesis** Danielle E Talbot, Bailey J Vormezeele, Garrett C Kimble, Dylane M Wineland, Daniel J Kelpsch, Michelle S Giedt, Tina L TootleAnatomy and Cell Biology, University of Iowa

Prostaglandins (PGs), locally acting lipid signals, regulate female reproduction, including oocyte development. However, the cellular mechanisms of PG action remain largely unknown. We hypothesize that one role of PGs during oogenesis is to regulate nucleolar activity. Supporting this hypothesis, PGs regulate nucleolar morphology across organisms. Changes in nucleolar morphology indicate altered function. Nucleolar functions, including ribosome biogenesis, are critical for producing viable eggs. Here we take advantage of the robust, *in vivo* system of *Drosophila* oogenesis to define the roles and downstream mechanism whereby PGs regulate the nucleolus. Loss of PGs results in striking changes in nucleolar morphology. This abnormal nucleolar structure is not due to a loss of ribosomal RNA (rRNA) transcription. Instead, loss of PGs results in increased rRNA transcription and overall protein translation. PGs modulate these nucleolar functions by tightly regulating nuclear actin. Specifically, multiple forms of nuclear actin, both monomeric and polymeric, are enriched in nucleoli during *Drosophila* oogenesis. Loss of PGs results in both increased nuclear actin and changes in its form. Increasing nuclear actin levels alone or co-reduction of both PGs and the nuclear actin export factor, Exportin 6, results in increased rRNA transcription. Together these data reveal PGs carefully balance the level and forms of nuclear actin to control the level of nucleolar activity required for producing fertilization competent oocytes.

373T **Loss of Nemp triggers a fertility-linked DNA damage checkpoint through chk2 and ATM** Yonit Bernstein[1], Andrea Jurisicova[2], Helen McNeill[31]Cell Biology, The Hospital for Sick Children, [2]Lunenfeld-Tanenbaum Research Institute, [3]Washington University School of Medicine

Whole-animal loss of dNemp (*Drosophila* Nuclear envelope membrane protein) causes sterility in both males and females. In mice, loss of Nemp1 leads to severe and early loss of fertility in females caused by a striking decrease in primordial follicle numbers, as well as disruption of developmental competence in fertilized embryos. The few follicles that develop in *dNemp* mutants display mislocalized Orb staining. Checkpoint protein-2 kinase (chk2) is a target of ataxia telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR), and are required for DNA damage-mediated cell cycle arrest. In *Drosophila,* downregulation of chk2 and ATM, but not ATR or p53 rescues *dNemp* follicle production, but not fertility. In mice, treatment of *Nemp1* mutant ovaries with chk2 chemical inhibitors significantly increases primordial follicle counts. Together, these data suggest that loss of Nemp triggers cell cycle arrest that contributes to preservation of reproductive quality.

374T **The bHLH-PAS transcriptional complex Sim::Tgo plays active roles in late oogenesis to promote follicle maturation and ovulation** Rebecca Oramas, Elizabeth Knapp, Jianjun SunPhysiology and Neurobiology, University of Connecticut

Across species, ovulation is a process induced by a myriad of signaling cascades that ultimately results in activation of proteolytic enzymes and degradation of the follicle wall to release encapsulated oocytes. Follicles need to become mature and gain ovulatory competency before ovulation. Unfortunately, the signaling pathways regulating follicle maturation are incompletely understood in *Drosophila* and other species. Our previous work showed that bHLH/PAS-family transcription factor Single-minded (Sim) likely plays important roles for follicle maturation downstream of NR5A-family nuclear receptor Ftz-f1 in *Drosophila.* Here, we explore the mechanism of Sim-regulated follicle maturation. We demonstrated that Tango (Tgo), a class-II bHLH/PAS-family transcription factor acts as a cofactor of Sim to promote follicle cell differentiation from stage 10 to 12. In addition, we discovered that re-upregulation of Sim in stage-14 follicle cells is also essential to promote ovulatory competency by upregulating octopamine receptor in mushroom body (Oamb), matrix metalloproteinase 2 (Mmp2), and NADPH oxidase (Nox), all of which are critical for successful ovulation. Together, our work indicates that the transcriptional complex Sim::Tgo plays multiple roles in late-stage follicle cells to promote follicle maturation and ovulation.

375T **Using RNAi to Identify *Enhancer of SD*** Aimee J. Smith, Janna R. McLeanBiology & Chemistry, Bethel University

Segregation Distorter, SD, is a system in *Drosophila melanogaster* that causes a deviation from normal patterns of Mendelian inheritance by preferentially transmitting the *SD* chromosome over the SD^+ homolog. This result is referred to as distortion. Distortion is only seen in male offspring, where the SD^+ sperm fail to develop properly. This phenomenon is thought to be caused by the *Sd* gene and several of its counterparts, including the *Enhancer of SD [E(SD)]. E(SD*) is known to be a key player in the *SD* system but its identity has yet to be discovered. Since *E(SD)* is a dominant gain of function

mutation, and since it is required for full distortion, we reasoned that knocking down mutant E(SD) product would suppress distortion. To test this method, we used an RNAi construct of *RanGAP* driven by the *bamGAL4dicer* construct provided by the Fuller lab. Our results show complete suppression of distortion. We are now using the same technique to test three genes that lie in the region of *E(SD*): *Spf45, CR40005,* and *CG4006.*

376T **How does Oskar assemble the *Drosophila melanogaster* germ plasm?** Anastasia Repouliou[1], John R. Srouji[1,2,3], Andrés E. Leschziner[2,4], Cassandra G. Extavour[1,21]Molecular and Cellular Biology, Harvard University, [2]Organismic and Evolutionary Biology, Harvard University, [3]BioNTech SE, [4]Cellular and Molecular Medicine, University of California, San Diego

Germ cells are uniquely capable of passing on genetic information to the next generation in sexually reproducing multicellular organisms. The *Drosophila melanogaster* protein Oskar is necessary and sufficient for the assembly of germ plasm, the specialised cytoplasm that confers germ line fate in the developing embryo. However, the molecular mechanism of Oskar-mediated germ plasm assembly remains unclear. The folded Oskar LOTUS domain forms homodimers and tetramers *in vitro*. Mutating specific residues in the interaction interfaces of LOTUS disrupts the *in vitro* LOTUS oligomerisation and its *in vitro* binding with Vasa, a key germ plasm protein. We hypothesise that Oskar LOTUS oligomerisation is relevant for the recruitment of functional germ plasm *in vivo.* I am testing this hypothesis by assessing the ability of these Oskar mutant proteins to recruit germ plasm *in vivo.* My approach is to ectopically express the mutant proteins at the anterior of developing oocytes and embryos. For each mutant, I quantify the ectopic enrichment of germ plasm components in oocytes and embryos and the existence and number of ectopic germ cell precursor cells in embryos. I anticipate that the results of my experiments will shed light on the molecular mechanism of Oskar as a nucleator, assembler, and localiser. We are thus leveraging the tractable *Drosophila* system to understand ubiquitous cell biology processes, namely the assembly and asymmetrical localisation of protein and mRNA in development.

377T **A cell-autonomous role for triglyceride lipase *brummer* in regulating lipid droplets and differentiation during *Drosophila* spermatogenesis** Charlotte F Chao[1], Yanina-Yasmin Pesch[2], Huaxu Yu[3], Maria Aristizabal[4], Tao Huan[3], Guy Tanentzapf[1], Elizabeth Rideout[11]Cellular & Physiological Sciences, University of British Columbia, [2]Nature Publishing Group, [3]Chemistry, University of British Columbia, [4]Queens University

Drosophila is a powerful model to study how lipids affect sperm development. Studies in flies showed that phosphatidylinositol and its phosphorylated derivatives influence spermatogenesis, and uncovered roles for membrane lipids and fatty acids in this process. Less is known about how neutral lipids, another major lipid group, affect sperm development. Neutral lipids include triglycerides and cholesterol esters, and reside within specialized organelles called lipid droplets (LD). LD are found in diverse cell types, and contribute to many cellular processes in nongonadal cells. Emerging evidence shows testis LD are present in normal and stressful contexts, genes associated with neutral lipid metabolism and LD have testis expression, and disruption of these genes causes subfertility. However, many questions remain about the spatial, temporal, and cell type-specific requirements for neutral lipids and LD during spermatogenesis. Here, we show that LD are present in early-stage somatic and germline cells within the *Drosophila* testis. We identified a role for triglyceride lipase *brummer* (*bmm*) in regulating testis LD, and show that whole-body and cell-autonomous loss of *bmm* leads to defects in sperm development. Because lipidomic analysis of *bmm* mutants revealed excess triglyceride accumulation, and spermatogenic defects in *bmm* mutants were rescued by genetically blocking triglyceride synthesis, our data suggest that *bmm*-mediated regulation of triglyceride influences sperm development. This identifies triglyceride as an important lipid that contributes to *Drosophila* sperm development, and reveals a key role for *bmm* in regulating testis triglyceride levels and spermatogenesis.

378T **Nucleoporin107 is a critical determinant of soma-germline communication, essential for ovarian development and function** Tgst Levi[1], Tikva Shore[1], Merav Yaffa Gold[1], Shira Leebhoff[1], Tzofia Bialistoky[1], Girish Deshpande[2], Offer Gerlitz[31]Developmental Biology and Cancer Research, Institute of Medical Research Israel-Canada, The Hebrew University- Faculty of Medicine, [2]Department of Molecular Biology, Princeton University, [3]Developmental Biology and Cancer Research, The Hebrew University

We recently identified a 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. Modeling of the human mutation in *Drosophila* resulted in female-specific infertility due to aberrant ovarian development, which to a large extent mimicks the human phenotype. Furthermore, selective RNAi-based knockdown of Nup107 indicated that Nup107 gonadal function is restricted to the soma. Our analysis demonstrated that Nup107

regulates essential soma-germline interactions in both larval and adult stages essential to establish and maintain ovarian differentiation and function. To elucidate the molecular underpinnings of Nup107 function, we carried out comparative transcriptome analysis on Nup107 mutant and control larval gonads. Among 82 differentially regulated candidate genes, we were intrigued by the down-regulation of the master sex determinant transcription factor *doublesex* (*dsx*). Functional analyses demonstrated that 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. We have also discovered 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. Importantly, the aberrant phenotypes induced by compromising either *Nup107* or *dsx* are reminiscent of BMP signaling hyperactivation. Remarkably, in this context, the metalloprotease AdamTS-A, a transcriptional target of both Dsx and Nup107, is necessary for the calibration of BMP signaling. As modulation of BMP signaling is a conserved critical determinant of soma-germline interaction, the sex- and tissue-specific deployment of Dsx-F by Nup107 seems crucial for the maintenance of the homeostatic balance between the germ cells and somatic gonadal cells.

379T **Sex chromosomes expression evolution in Drosophila spp. spermatogenesis** Camila C Avelino[1], Carolina A Mendonça[2], Gabriel N Goldstein[1], Maria D Vibranovski[31]Department of Genetics and Evolutionary Biology, University of São Paulo, [2]Harvard T. H. Chan School of Public Health, Harvard University, [3]School of Mathematical and Natural Sciences, New College for Interdisciplinary Arts and Sciences, Arizona State University

Meiotic Sex Chromosome Inactivation (MSCI) is a transcriptional silencing of sex chromosomes in the early stages of male meiosis. A recent publication showed the presence of downregulation on the D. melanogaster X chromosome and also it was observed a similar pattern for the ancient X chromosome, commonly called dot. However, the impact of MSCI on the Drosophila genus is unknown. To investigate the evolutionary panorama of MSCI, we used RNA-seq data of the three main phases of spermatogenesis (mitosis, meiosis, and post meiosis) which was obtained according to the Vibranovski et al. (2009) technique of dissection. The isolation was performed in four species of Drosophila genus: D. melanogaster, which the mechanism was broadly studied and we use to compare with D. simulans, a close specie of D. melanogaster; D. willistoni, which has a neo-X chromosome and it belongs to a sister group and D. mojavensis, a specie from an external group. Although the differences in morphology and RNA quantity for each species, we found similar expression profiles of spermatogenesis for all transcriptomic data using k-means clustering. Moreover, an underrepresentation of overexpressed genes on the X and dot chromosomes in meiosis compared to mitosis was found in three of these four analyzed species. Interestingly, D. willistoni Neo X shows a similar under expression in meiosis, suggesting a presence of MSCI, as observed in muller A of D.melanogaster. Overall, we found that MSCI is generally preserved in the genus Drosophila, including when it comes to X chromosomes of different ages.

380T **Identifying Recombination Nodule Proteome via TurboID-based Proximity Labeling** Oscar B Bautista, Elizabeth Melton, Nicole CrownBiology, Case Western Reserve University

During meiosis, recombination between homologous chromosomes ensures genetic diversity among haploid products, but errors in this mechanism are a major source of human infertility. Recombination events, initiated by enzymatically directed double-strand breaks (DSBs), can result in crossovers (COs), involving reciprocal exchanges between homologues, or noncrossovers, where a portion of DNA is copied from the donor homologue without altering it. To promote the formation of COs, several early acting proteins such as the Msh4/5 complex function to stabilize CO intermediates while late acting endonucleases Mlh1/3 resolve double Holliday junctions. *Drosophila* lacks homologs of many of these pro-crossover proteins and seems to functionally replace some of these important complexes with other combinations of proteins. However, *Drosophila* expresses a Zip3 homolog, Vilya, that has been shown to be required for DSB formation and is currently the only known component of fly recombination nodules, electron-dense protein complexes that form around CO sites. Vilya encodes a E3 ubiquitin ligase domain and is hypothesized to be a post translational regulator for meiotic proteins at these recombination nodules. Currently, it is unknown which target meiotic proteins Vilya regulates and what other proteins exist at recombination nodules. To address these gaps, we have generated fly lines that contain an engineered biotin ligase TurboID conjugated to Vilya and Rec, which functionally replaces the Msh4/5 complex in flies, to perform proximity labeling. Utilizing these lines, we have confirmed that these endogenously tagged TurboID proteins biotinylate proteins along the SC, the zipper-like protein structure that forms during meiosis to keep homologous chromosomes together. Next, we will be using this system to identify the biotinylated proteins that were shown through confocal microscopy through mass spectrometry. The protein-protein interactions uncovered will also provide insight into the complex regulatory mechanisms that are required to form COs.

381T **Cytological characterization of *mei-P26*^{1} and its effect on meiotic recombination** Erica Berent, Nicole CrownCase Western Reserve University

Meiosis is a developmentally regulated process that is essential for reproduction in many organisms. In order to identify key regulatory steps in the developmental control of meiosis, we are using mutations in the gene *meiosis P26* (*mei-P26*), which plays an important role in meiosis, germline differentiation, and spermatogenesis. One well studied mutant allele of this gene, *mei-P26*^{fs1}, is characterized by a lack of differentiation of stem cells in the germarium. A different mutant allele, *mei-P26*^{1}, shows typical stem cell differentiation, but still displays a high level of nondisjunction (17.3% on chromosome X and 8.4% on chromosome 4) and a large reduction in meiotic crossovers (30-80% reduction) (Page et al 2000). Preliminary data suggests that it may affect the formation and structure of the synaptonemal complex (SC) (Page and Hawley 2000), which is required for the formation of crossovers. In order to further characterize this mutant phenotype, we are cytologically characterizing defects in SC and crossover formation and genetically characterizing the defects in crossover formation. With this data, we will be able to gain a better understanding of *mei-P26*^{1}'s effect on the SC and the consequences it has in meiotic recombination.

382T **Maternal organelle contribution to offspring germline health** Jay S Goodman, Emily Dawson, Fabian Schulte, Ruth LehmannWhitehead Institute for Biomedical Research

Organelles are inherited from cell to cell to maintain organismal health. In contrast to somatic tissues, the germline poses a challenge to prevent lipid and protein organelle contents from deterioration as they are inherited between generations. Intriguingly, quality control events naturally occur in oogenesis to prevent damaged organelles from being inherited by the egg. However, eggs can be arrested for a prolonged period prior to fertilization, allowing organelle long-lived proteins to accumulate damage before embryogenesis. Upon egg fertilization, two-thirds of maternally deposited transcripts are degraded to initiate the maternal to zygotic transition in the developing embryo, while many maternally deposited organelle proteins persist during the onset of zygotic genome activation. The degree in which maternally deposited organelles perdure and exchange with zygotic organelles during germline development is not well understood. Furthermore, germ cells are largely quiescent in the embryo and undergo a limited number of cell divisions before progeny are mature enough to reproduce. This raises the intriguing question if additional quality control pathways protect the progeny's germ cells from accumulating maternal organelle long-lived proteins to prevent organelle deterioration from occurring in the germline. Through molecular and genetic analysis, I will investigate how maternal organelles deposited in the egg of Drosophila influences offspring germline health. I have found that germ cells contain maternally deposited nucleoporin condensates and plan to characterize the fate of these condensates throughout germline development. Next, I have found that aged eggs exhibit a 20% reduction in germ cells and will test how perturbing egg health impacts the quality of maternally deposited nucleoporins. Finally, I will globally identify additional perduring maternal organelle proteins using a pulse-chase amino acid labeling approach and assess how these additional organelle long-lived proteins impact germline health. The proposed experiments will highlight how maternally deposited long-lived proteins impact organelle function in the germline of ensuing progeny. In addition, this research will demonstrate how organelle continuity is maintained between generations and aim to reveal specialized quality control pathways in the germline.

383T **Recent functional divergence of testis-specific HMG-box-containing genes (tHMGs) in Drosophila** Ching-Ho Chang, Harmit S. Malik, Isabel Mejia NatividadFred Hutch

Testes recruit extensive stage- and cell-specific gene expression to produce specialized gametic cells, *e.g.*, sperm. This is often achieved by a distinct set of transcription factors (TFs) that share homology with ubiquitous TFs. To study how transcription factors acquired testis-specific function, we focus on a young testis-specific gene, tHMG, in *Drosophila*. tHMG gene has arisen in the higher Diptera from the duplication of a ubiquitous TF (HmgD or HmgZ). It harbors a DNA-binding high mobility group domain, which is shared across many *Drosophila* protamines, and thus is considered an early stage of evolution toward protamines. tHMG was tandemly duplicated in *D. melanogaster*, and the two copies (tHMG1 and tHMG2) have been shown to harbor different expression and cytological patterns in spermatogenesis. Consistent with the observation, we showed that only tHMG1, but not tHMG2, is under positive selection, and its knockdown reduces fertility, suggesting their divergent functions. We also found that tHMG has many duplications in other species, especially in the *D. simulans* clade species, which only diverged from *D. melanogaster* 3 million years ago. In these species, the tHMG gene duplicated and dramatically expanded to up to fifty copies on the X chromosome. We suspect the amplification of X-linked tHMG genes might be driven by the conflict between sex chromosomes. Our hypothesis is sex chromosomal tHMG expansions can act selfishly to kill sperm without themselves to increase their

frequency in the offspring known as meiotic drive. In contrast, ancestral tHMG genes have been retained on autosomes to act as suppressors. We are generating CRISPR knockouts and transgenic flies to test this hypothesis and identify the regions determining the functional differences between these recently diverged duplicates. Our study will highlight how new testis-specific TFs acquired roles in sperm development and further shed light on the evolutionary forces shaping spermatogenesis.

384T **OVO binds and regulates transcription start sites of genes encoding major egg and maternal functions including axis specification, vitelline membrane formation, and egg activation** Leif Benner[1,2], Savannah Muron[1], Brian Oliver[11]Section of Developmental Genomics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, [2]Department of Biology, Johns Hopkins University

Sexual reproduction has evolved as a fundamental mechanism for the propagation of life. Each sex must initiate a genetic program instructing the germline to differentiate into the correct sex-specific gamete. OVO is required for female, but not male germline viability, and has been shown to positively regulate its own expression, as well as a downstream target, *otu*, by binding to the transcriptional start site (TSS). To find additional OVO targets in the female germline, we performed ChIPseq on OVO to determine its genome wide occupancy, as well as RNAseq on ovaries from flies with partial loss-of-function alleles to analyze which genes respond to the presence of OVO. Our ChIP analysis showed that 1,583 out of a total 3,094 (51%) significant OVO ChIP peaks overlapped with a gene TSS, suggesting that OVO functions at the core promoter. Motif enrichment analysis on OVO ChIP peaks identified a 5`-TAACNGT-3` motif (similar to the *in vitro* OVO DNA binding motif described) often overlapping the CA dinucleotide that is favored by RNA Polymerase II. Integrated analysis showed that 457 genes that are bound and upregulated by wildtype OVO are known to be maternally loaded into eggs and early embryos. These include genes involved in anterior/posterior/germ plasm specification (*bcd*, *exu*, *swa*, *osk*, *nos*, *pgc*, *gcl*), vitelline membrane formation (*fs(1)M3*, *fs(1)N*, *clos*), and egg activation (*png*, *plu*, *gnu*, *CycB*, *wisp*). This suggests that OVO is a master regulator of oogenesis. To determine if this was due to the particular core promoter, we placed a 10x UAS upstream of a typical 1,300 bp OVO core promoter (creating a new vector, UASo), and found that this promoter can only activate transcription in the presence of GAL4 in the female germline and not in other somatic tissues such as the eye. This likely indicates that OVO carries out its ability to regulate transcription by binding to the TSS and thus cooperates directly with components of the RNA polymerase complex to direct female-specific germline transcription.

385T **Bourbon interacts with Otu and promotes the expression of Sxl in the *D. melanogaster* female germline** Marianne Mercer, Michael BuszczakMolecular Biology, UT Southwestern

Sexually reproducing species exhibit a wide variety of mechanisms to establish male and female identity at the cellular level. In *D. melanogaster, sex lethal (sxl)* is the master switch of sex determination in somatic cells; however, the process of sex determination in the germline remains poorly understood. Previous studies show that *ovarian tumor (otu*) acts genetically upstream of and promotes the expression of Sxl in the female germline, which ultimately leads to a female fate. How Otu protein promotes Sxl expression is less clear. Through IP-mass spec analysis we find that Otu physically interacts with Bourbon (Bbn), a previously uncharacterized protein. Bbn shares structural similarities with human MYCBP. OTUD4 and MYCBP, human homologues of Otu and Bbn respectively, have been shown to physically interact in human cells indicating the relationship between these proteins may be conserved across species. *bbn* null mutant females exhibit defects in germ cell differentiation with agametic ovarioles and cystic germline tumors. These germline tumors do not express Sxl protein and resemble *sxl* sterile mutants, displaying expanded and overlapping expression of Bam and Nanos. Transgenic expression of *sxl* can partially rescue this phenotype. Because the phenotypes and localization pattern of *bbn* are strikingly similar to those of *otu* and they physically interact, we are testing how Bbn impacts the ability of Otu to promote Sxl expression. We hypothesize that Otu, the founding member of a family of deubiquitinases, deubiquitinates Sxl to prevent its degradation by the proteasome. We have found Sxl levels are regulated by the proteasome and are testing the direct ubiquitination of Sxl. Future experiments will help us determine if Otu deubiquinates Sxl and if Bbn regulates Otu expression levels, localization, substrate specificity, or catalytic activity.

386T **Sar1, a GTPase involved in COPII vesicle trafficking, is critical for *Drosophila* oogenesis** Makayla C Gomperts, Julie A MerkleBiology, 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 15 others becoming supporting cells, or 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, CRISPR/Cas9 gene editing was used to generate indels in the genomic region encoding the C-terminus of *Sar1*. Plasmids encoding gRNAs were injected into embryos to establish lines that were confirmed by sequencing. Additionally, using RNAi lines and a variety of germline-specific Gal4 drivers, the knockdown phenotype of *Sar1* was also analyzed. Available CRISPR alleles were also utilized to determine loss of function phenotypes of *Sar1* by employing the TRiP-KO and WKO systems. Excitingly, several RNAi and CRISPR lines exhibit severe defects in oogenesis, suggesting that *Sar1* is critical for ovary development and egg production. Investigating the role of *Sar1* in COPII vesicle trafficking will allow for better understanding of the mechanism of oocyte fate determination in *Drosophila*.

387T **The *Drosophila* Estrogen-Related Receptor acts as a central regulator of oogenesis** Sophie A Fleck[1], Katherine Beebe[2], Jason M Tennessen[1], Lesley N Weaver[11]Department of Biology, Indiana University Bloomington, [2]Department of Human Genetics, University of Utah

Reproduction is an energetically intensive process that requires the systemic coordination of intrinsic physiological processes with extrinsic cues such as nutrient availability and environmental stress. As a result, reproductive adults rely on a series of conserved metabolic regulators to achieve a balance between energy production and storage, biosynthesis, and interorgan metabolic trafficking. In this regard, the *Drosophila* Estrogen-Related Receptor (dERR), the sole fly ortholog of the ERR family of nuclear receptors (NRs), represents an intriguing candidate for integrating metabolism and physiology with reproductive development. Previous studies of *dERR* demonstrate that this NR activates stage-specific metabolic programs. For example, dERR activation during the latter half of embryogenesis up-regulates glycolysis and the pentose phosphate pathway (PPP), thus establishing a biosynthetic metabolic state that promotes larval growth. Similarly, dERR is required in adult males to coordinate glycolytic metabolism with lipid synthesis and within the testis to regulate spermatogenesis gene expression and testis formation. These observations suggest that dERR is a global regulator of developmental metabolism within the fly and raise the possibility that this NR contributes to other developmental processes during the life-cycle. To determine the requirement of dERR activity in adult females, we are using heat shock driven FLP-FRT recombination to conditionally knockout *dERR* and assay for defects in oogenesis processes and metabolic changes. Global knockout of *dERR* in adult females significantly reduces egg laying and causes morphological defects of the ovary (smaller size, opaque anterior region, and yellowing of mature egg chambers) indicating that multiple oogenesis processes may be affected. We are currently determining whether distinct steps of oogenesis that are sensitive to physiological changes such as germline stem cell maintenance and proliferation, follicle survival, and vitellogenesis are perturbed in the absence of *dERR*. Furthermore, dERR is a known transcriptional regulator of glycolytic enzymes and metabolic enzymes in the PPP. Interestingly, the PPP in the germline was shown to alter sugar appetite (the primary nutrient fueling the PPP) through regulation of fat-body-secreted satiety factor. Therefore, we are currently testing the hypothesis that dERR acts upstream of the PPP in the germline and potentially peripheral tissues to influence the energetically intensive process of oogenesis.

388T **The role of the linker protein, Moesin, in the germline of the developing egg chamber** Izabella C Jordan[1], Lindsay Lewellyn[2], Allison Foster[11]Butler University, [2]Biology, Butler University

Egg development, or oogenesis, involves cell division, changes in cell shape, and cell migration; all these processes require dynamic changes in the cell membrane and the underlying actin cytoskeleton. Each fly egg develops from a structure called an egg chamber, which consists of a cluster of germ cells (nurse cells and the oocyte) surrounded by a layer of somatic follicle cells. Moesin is an important linker protein that connects the actin cytoskeleton to membrane proteins, and in the developing egg chamber, Moesin localizes to both the germ cells and somatic cells. Like other ERM proteins, Moesin contains a central alpha helical region which separates the N-terminal FERM domain and the C-terminal actin-binding domain. Activation of Moesin requires phosphorylation of a threonine residue within its actin binding domain, which disrupts an intramolecular interaction with the N-terminal domain. Active Moesin is required to promote border cell migration, and *moe* germline mutants show defects in the actin cortex and establishment of polarity in the oocyte. Within the germ cells, Moesin localizes to nurse cell membranes, and the active, phosphorylated form is enriched around the germline ring canals. Because ring canal stability and growth likely requires strong, yet dynamic connections between the membrane and actin cytoskeleton, we hypothesized that Moesin could also be required to

regulate ring canal size and/or stability. To test this hypothesis, we have used the dominant female sterile technique to generate homozygous mutant germline clones for each of three different *moe* mutations. We have monitored ring canal size as well as the size of the mature eggs that develop from these egg chambers. In addition, we have analyzed ring canal size and structure in egg chambers expressing either a phosphomutant (T559A) or phosphomimetic (T559D) form of Moesin in the germline. In the future, we hope to determine how Moesin activity is regulated in the germline.

389T **Roles of *Drosophila* DDX42 on ovarian growth and oocyte development in the response to stress** LUCIA BETTEDINICHD, NIH

The pathways that monitor nutritional availability and stress play a central role in the regulation of the meiotic program and growth during oogenesis. Target of Rapamycin Complex 1 (TORC1) is a critical node of an intricate network of diet-dependent pathways that modulates metabolism and promotes growth in response to multiple upstream inputs. Compromising TORC1 activity in the female germline leads to a reduction of ovarian growth and a block of oocyte development. Conversely, mutations in inhibitors of TORC1 result in premature ovarian failure in mice. Our lab completed a large-scale RNAi-based screen to identify genes that when silenced, suppress the ovarian phenotypes of mutants with low TORC1 activity. From this screen we identified a novel gene encoding for the putative RNA-binding helicase (DDX42). We generated CRISPR-Cas9 *ddx42KO* null mutant flies and rescued the growth deficit of TORC1 mutants, thus confirming the results from our screen. *ddx42KO* null homozygous mutants are infertile, they exhibit autolysosomal abnormalities and they are insensitive to nutritional starvation further implying that DDX42 regulates TORC1 activity, growth and stress response during oogenesis. In parallel to our work in *Drosophila*, we generated CRISPR-Cas9 *ddx42KO* null mutant HeLa cells to determine the transcriptome change and splicing pattern affected by the absence of DDX42. Interestingly, we found that genes involved in the stress response are significantly downregulated in the mutant HeLa cells compared to wt HeLa cells upon starvation thus suggesting a critical role of DDX42 in the regulation of stress-related mRNAs. We found that sestrin (a negative regulator upstream of TORC1) is greatly reduced at transcriptional level in the mutant cells upon starvation. In *Drosophila*, *sestrin* mutants fails to activate autophagy in the ovary and are insensitive to starvation thus recapitulating some of the phenotypes observed in the *ddx42KO Drosophila* mutants. By overexpressing a tagged version of DDX42 in the ovary, via pull-down and Mass Spec experiments we determined that DDX42 associates with multiple components of the spliceosome. Since upon the introduction of a stress, DEAD box and spliceosomal proteins modulate the gene expression at post-transcriptional level in specialized membraneless compartments (or germline ribonucleoprotein (RNP) granules) of the *Drosophila* ovary, we are currently investigating whether DDX42 plays a role in the signaling pathway that connects mRNA processing and the response to nutritional stress and developmental inputs during oogenesis.

390T **TRPM's role in protein phospho-regulation during Drosophila egg activation** Jonathon M. Thomalla[1,2], Mariana F. Wolfner[11]Molecular Biology and Genetics, Cornell University, [2]Biomedical Sciences, Cornell University

To ensure successful initiation of embryonic development, a mature oocyte must turn on the programs needed for zygotic development. This developmental transition, called egg activation, occurs in the absence of transcription. Its events, including completion of stalled meiosis and translation of maternal mRNAs, require a rise in calcium levels in the oocyte. Phosphorylation states of hundreds of maternally-provisioned proteins change during egg activation, prompting the hypothesis that their phospho-states act as 'on' or 'off' switches for the events of egg activation. We previously showed that the calcium-activated phosphatase calcineurin, which is essential for egg activation, regulates a subset of phospho-changes. Calcineurin-mediated phospho-changes include cell cycle and translation regulators, linking the protein phosphorylation changes and their consequences to the calcium rise. The calcium rise in Drosophila oocytes begins with local calcium entry mediated by the TRPM cation channel, and spreads across the oocyte in a wave. To determine the extent of its effects on maternal proteins, we compared the phosphoproteomes of TRPM knockdown (KD) oocytes and activated, unfertilized eggs to those of controls. Surprisingly, our preliminary analysis of the mass spectrometry data indicates that many phospho-changes, including some shown to be calcineurin dependent, occur normally in TRPM KDs; for example, the translational regulatory protein Gnu and M-phase regulator Endos undergo their normal dephosphorylation events. However, the APC-regulator Fzy does not undergo its normal calcineurin-mediated dephosphorylation event in activating TRPM KD eggs. We are exploring compensatory and temporal mechanisms that may explain the mild effect we see of TRPM KD on the phosphoproteome, with the goal of disentangling the players and timescale for egg activation and any 'fail safe' mechanism(s) that eggs utilize to ensure robust early development.

391F **Effects of Yolk Proteins During Egg Activation in Drosophila** Clarisa Cristobal[1], Jonathon M Thomalla[2], Kathleen E Gordon[2,3], Brian P Lazzaro[3], Mariana F Wolfner[21]Molecular Biology and Genetics and Entomology, Cornell

Univeristy, [2]Molecular Biology and Genetics, Cornell University, [3]Entomology, Cornell University

In Drosophila melanogaster, egg activation (resumption of meiosis and translation by a mature oocyte) is triggered by mechanical or osmotic pressure leading to calcium influx. The first visible step is swelling of the oocyte due to hydration. Immediately afterwards, a calcium wave initiates from the oocyte pole(s) through the action of TRPM channels in the membrane; the calcium rise is needed for egg activation. In 2021, Tanaka et al. reported that eggs lacking Yolk Proteins or yolk protein receptors do not rehydrate and have reduced fertility. We would like to determine the role of yolk proteins or their receptors in the initiation of the calcium influx and wave. We have begun by quantitatively assessing the extent to which oocytes fail to hydrate without yolk proteins and or their receptors. After dissecting them from y*olk protein*$^{1-3}$, *yolkless*$^{16-2}$, and *yolkless*$^{16-6}$ females, we placed oocytes into a hypotonic activation buffer and examined the extent of swelling. We found a significantly decreased hydration rate in all three mutants compared to controls, consistent with the report from Tanaka et al. We are now testing whether the failure of these oocytes to swell/hydrate affects the initiation of progression of their calcium wave.

Tanaka T, Tani N, Nakamura A (2021) Receptor-mediated yolk uptake is required for oskar mRNA localization and cortical anchorage of germ plasm components in the Drosophila oocyte. PLoS Biol 19(4): e3001183. https://doi.org/10.1371/journal.pbio.3001183 392F **Identification and Analysis of Key Transcription Factor Genes in Differential Spermatogenesis in *Drosophila pseudoobscura*, a Sperm Heteromorphic Species** Fiona Messer, Helen White-CooperBiosciences, 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.

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.

Prior to this work, little was known regarding the specific molecular and developmental processes that produce multiple sperm morphs in *D. pseudoobscura*, although it was known primary spermatocytes within a single cyst all generate the same sperm morph. We hypothesised that transcriptional variation would be present between sub-sets of primary spermatocyte cysts, and that this would underpin sperm morph differentiation. RNA-seq analysis of manually isolated individual spermatocyte cysts, followed by cluster analysis, confirmed transcriptional differences between three classes of cysts, prior to the onset of meiosis. 1000 genes were differentially expressed between primary spermatocyte classes. Potential functions of differentially expressed genes (inferred by analysis of their *D. melanogaster* orthologues) included transcription factors, meiosis regulators, and genes required for spermatogenesis and spermiogenesis, including axoneme assembly and mitochondrial fusion.

Among putative transcriptional regulators, we validated, by *in situ* hybridisation, differential expression of some components of the testis meiotic arrest complex (tMAC), consistent with variant tMAC composition between cysts. We also validated differential expression of the tMAC regulator *kumgang (kmg)*. A transgenic line expressing Kmg-GFP fusion revealed that the Kmg protein is high in some late spermatocyte cysts, but low or absent from others and we propose that this contributes to morph differentiation. In *D. melanogaster*, *kmg* acts as both a transcriptional repressor and an activator (of different target genes), ongoing ChIP-seq analysis will reveal how *kmg* association with chromatin correlates with differences in gene expression between spermatocyte classes.

393F **Heterozygous Inversion Breakpoints Suppress Meiotic Crossovers by Altering Recombination Repair Outcomes** Haosheng Li[1], Erica Berent[1], Savannah Hadjipanteli[1,2], Miranda Galey[3,4], Danny E Miller[3,4], Nicole Crown[11]Case Western Reserve University, [2]University of Houston, [3]University of Washington, [4]University of Washington and Seattle Children's Hospital

Heterozygous chromosome inversions suppress meiotic crossover (CO) formation within an inversion, potentially because they lead to gross chromosome rearrangements that produce inviable gametes. Interestingly, COs are also severely reduced in regions nearby but outside of inversion breakpoints even though COs in these regions do not result in rearrangements. Our mechanistic understanding of why COs are suppressed outside of inversion breakpoints is

limited by a lack of data on the frequency of noncrossover gene conversions (NCOGCs) in these regions. To address this critical gap, we mapped the location and frequency of rare CO and NCOGC events that occurred outside of the dl-49 chrX inversion in D. melanogaster. We created full-sibling wildtype and inversion stocks and recovered COs and NCOGCs in the syntenic regions of both stocks, allowing us to directly compare rates and distributions of recombination events. We show that COs are completely suppressed within 500 kb of inversion breakpoints, are severely reduced within 2 Mb of an inversion breakpoint, and increase above wildtype levels 2-4 Mb from the breakpoint. We find that NCOGCs occur evenly throughout the chromosome and, importantly, occur at wildtype levels near inversion breakpoints. We propose a model in which COs are suppressed by inversion breakpoints in a distance-dependent manner through mechanisms that influence DNA double-strand break repair outcome but not double-strand break location or frequency. We suggest that subtle changes in the synaptonemal complex and chromosome pairing might lead to unstable interhomolog interactions during recombination that permits NCOGC formation but not CO formation.

394F **Meiotic Crossover Patterning: Understanding the *D. melanogaster* Centromere Effect** Nila M Pazhayam[1], Jeff J Sekelsky[21]Genetics and Molecular Biology, University of North Carolina at Chapel Hill, [2]Biology, University of North Carolina at Chapel Hill

Crossing-over between homologous chromosomes is a critical part of meiosis that prevents aneuploidy by promoting proper chromosome segregation. By facilitating accurate disjunction of homologs, crossing-over forestalls miscarriages and chromosomal disorders such as Down syndrome, the risk of which increases with maternal age. Meiotic crossovers (COs) are formed from programmed double-strand breaks (DSBs) that undergo homologous recombination. Although the DSBs that initiate crossing-over are distributed throughout the chromosome, intricate patterning governs where COs are placed. Three types of patterning events have been observed, one of which is the centromere effect (CE) that ensures CO exclusion in the regions surrounding the centromere. The CE is crucial to the meiotic cell, as centromere-proximal COs increase risk of nondisjunction. Despite its importance, the mechanisms behind the CE are poorly understood. To address this gap in knowledge, I am investigating the mechanisms underlying the CE using *Drosophila* as a model system. Pericentric heterochromatin in *Drosophila* is not homogenous and is instead divided into two classes: the heterochromatin closer to the centromere (alpha heterochromatin) consists of highly repetitive satellite arrays, while that adjacent to euchromatin (beta heterochromatin) is less repetitive with some amount of unique sequence. Recent work from our lab has shown that the CE in *Drosophila* manifests as a complete exclusion of COs in alpha heterochromatin, and we hypothesize that this could be due to an absence of DSBs in this region. To test this, I am studying differences in the number and positioning of DSBs in flies with heterochromatin defects, as compared to wild type. Another avenue I am investigating is the sensitivity of the CE to total repetitive sequence content. Previously published data suggest that centromere-proximal CO rates should increase with increasing repetitive sequence content. However, my results indicate that changes in repetitive sequence content do not influence centromere-proximal CO frequencies at a genome-wide level, suggesting that although pericentric CO frequencies have been shown to be affected by local changes in repetitive sequences, global changes in repetitive sequence content do not seem to exert a trans-effect on the CE. Through these and other methods, my overarching goal is to gain further understanding of the mechanisms behind the *Drosophila* centromere effect.

395F **Nemp, a conserved IDR containing nuclear transmembrane protein, is essential for oogenesis** Ruichen C Cao[1], Yonit Tsatskis[2], Helen McNeill[11]Developmental Biology, Washington University in St. Louis, [2]Cell biology, The Hospital for Sick Children

Nuclear envelope membrane protein (Nemp) is a highly conserved multipass transmembrane protein that localizes to the inner nuclear membrane. Though Nemp is ubiquitously expressed, one of the most striking phenotypes of *Nemp* genetic null is sterility. This dramatic loss of fertility is conserved across flies, female mice, zebrafish, and worms. *Nemp* null flies are completely infertile, with shrunken ovaries that are incapable of producing eggs. Preliminary data shows NanosGal4 knockdown of Nemp in the ovary leads to almost total loss of early egg chambers, while BamGal4 driven knockdown has no apparent effect, indicating that Nemp functions in early oocyte development. The reasons for Nemp's tissue specific phenotype, as well as the mechanism by which Nemp functions to preserve fertility, is unknown. 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 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 will use *Drosophila* to explore some of these questions.

396F **Searching for the female receptor for the *D. melanogaster* seminal fluid protein ovulin.** Mengye Yang[1], Melissa White[1], Jennifer Apger-McGlaughon[1], Geoffrey Findlay[2], Mariana Wolfner[11]Cornell University, [2]College of the Holy Cross

Males transfer ~300 seminal fluid proteins (Sfps) along with sperm to the female reproductive tract during copulation. Sfps cause female post-mating responses, both behavioral and physiological, for optimal reproductive success. Although female molecules must interact with Sfps to facilitate these processes and affect reproduction, how this occurs is not understood. Ovulin is an Sfp that manipulates octopaminergic signaling, resulting in a short-term increase in ovulation. Ovulin likely interacts with a receptor within the mated female to stimulate growth by octopamine-producing Tdc2 neurons. Therefore, identifying the female's ovulin receptor (OvR) would significantly advance our understanding of the mechanism of ovulin's action and guide future investigations into Sfp actions, including in human fertility.

We performed two evolutionary rate co-variation screens to identify OvR candidates. We narrowed down the candidate list to twelve GPCRs, based on their effect on egg-laying and/or their expression pattern. Genetic analysis of ovulation rate in female flies knocked down for candidate receptors revealed candidate receptors with ovulin-dependent (OvR) or ovulin-independent roles in ovulation. Additional assays, including membrane-anchored split-ubiquitin yeast two hybrid assays, in vitro TANGO assay, molecular evolution analysis, and multimer structure prediction, we have narrowed the field to 3-4 top OvR candidate receptors.

397F **Tao kinase as a potential upstream regulator of the Misshapen kinase in the germline of the developing egg chamber** Abby Heilman, Jack Paras, Lindsay LewellynButler University

Intercellular bridges are an essential structural feature of developing eggs and sperm in many organisms, but the pathways that regulate their formation, stability, and growth are not fully understood. The developing egg chamber provides an excellent model system to study intercellular bridges. Within the egg chamber, the oocyte is connected to the supporting nurse cells through intercellular bridges called ring canals that allow the transfer of essential materials during egg development. We have previously shown that the Ste20 kinase, Misshapen (Msn), localizes to these germline ring canals and regulates their size and stability; however, it is not known how Misshapen is localized to the ring canals or how its activity is regulated during oogenesis. Here, we test the hypothesis that the Tao kinase phosphorylates and activates Msn in the germline to regulate the size and stability of the ring canals. Our preliminary data suggest that depletion of Tao in the germline alters ring canal size and the size of the mature eggs that are produced. In addition, Western blot analysis supports a role for Tao in phosphorylating Msn. In the future, we hope to further explore the role that Tao plays in regulating the localization and activity of Msn in the germline.

398F **A Mutational Approach to Analyze RNA Helicase Me31B's *in vivo* Working Mechanism in *Drosophila* Fertility and Germline Development** Evan Kara[1], Aidan McCambridge[1], Megan Proffer[1], Carol Dilts[1], Brooke Pumnea[1], John Eshak[1], Korey A Smith[1], Isaac Fielder[1], Dominique A Doyle[2], Bianca M Ortega[2], Yousif Mukatash[1], Noor Malik[1], Ammaar R Mohammed[1], Deep Govani[1], Matthew G Niepielko[2], Ming Gao[11]Indiana University Northwest, [2]Kean University

RNA helicase Me31B (DDX6) is a conserved germ granule protein that plays essential roles in germ *Drosophila* granule assembly, germline RNA metabolism, and germline cell development. However, Me31B's working mechanism remains mostly unclear. To study this, we mutated key functional domains/motifs of Me31B to analyze their roles in the protein *in vivo*. The domains/motifs include the three ATPase/helicase activity motifs (DEAD motif, DVLARAK motif, and HRIGR motif), the two RecA-like domains (N-terminal and C-terminal domains), and the FDF-binding motif (interacts with Tral protein's FDF motif). We used the CRISPR gene-editing technique and generated *Drosophila* strains mutant for the above motifs via point mutations, multi-point mutations, or truncation mutations. Then, the mutants were subjected to organism-, tissue-, and molecular-level assays to reveal the mutations' phenotypic effects on *Drosophila* fertility and germline development. At the organism level, all six mutations have a negative influence on fertility and/or embryo hatchability. Particularly, mutations in the three ATPase/helicase motifs cause dominant female sterility. At the tissue level, microscopic examination showed that the mutated Me31B proteins have different protein expression levels and/

or subcellular localization in the egg chambers than the wild-type proteins. At the molecular level, we observed that the ATPase/helicase mutants showed a significantly altered level of germline mRNAs like *nos* and *osk* mRNAs. Our study shows that Me31B plays an important role in *Drosophila* fertility and germline development, and the protein's functional motifs (ATPase/helicase motifs, N-terminal, C-terminal, and FDF binding motifs) respectively contribute to the protein's activities in regulating germline mRNAs, cellular expression and localization, and interaction with partner proteins.

399F **Protein Dynamics in the Early Drosophila Embryo** Chloe Briney, Emma Larkin-Gero, Jesslyn Henriksen, Olivia RisslandUniversity of Colorado, Anschutz Medical Campus

Prior to zygotic genome activation, the early Drosophila embryo proteome consists mainly of maternally deposited proteins. A key step of the maternal-to-zygotic transition (MZT) is 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 E2 Kondo and CTLH E3 complex. However, there are many other maternally deposited proteins in the early embryo that are destroyed by unknown mechanisms. Here, we have focused on the destruction of BicC because it is a maternally deposited RNA binding protein, essential for patterning in the early embryo, and robustly destroyed in the early MZT. Like ME31B-Cup-TRAL, BicC degradation depends on Pan Gu activity and translation, suggesting that an E2 or E3 must be translationally upregulated during the MZT in order for BicC to be cleared; however, unlike ME31B-Cup-TRAL, BicC degradation is not mediated by Kondo and the CTLH complex. This example of protein degradation in the early embryo is controlled by a new mechanism and so highlights the exciting complexity of mechanisms controlling maternal protein degradation during the MZT.

400F **Female germline OVO expression is eternal** Savannah Muron[1], Leif Benner[1,2], Brian Oliver[11]National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, [2]Department of Biology, Johns Hopkins University

In most organisms, continuity of life requires creation of sex-specific gametes. The initial choice between egg and sperm production is poorly understood. OVO is a good candidate for this early choice, as it is required for karyotypically female germ cell viability but has no known function in the male germline. *ovo* autoregulation involves two antagonistic isoforms, OVO-A and OVO-B, with OVO-A acting as a repressor and OVO-B acting as an activator of *ovo* transcription. To visualize OVO expression and localization during germline development and oogenesis, we made a series of tagged *ovo* alleles by CRISPR. We placed a 3x-FLAG-HA peptide sequence at the N-terminus of OVO-A and OVO-B as well as the shared C-terminus of both, thus allowing us to tag different isoforms with three different engineered alleles. Staining adult ovaries of flies bearing these different alleles revealed the presence of nuclear OVO in the stem cells and mitotic germ cells in the germarium and the differentiating germ cells. C-terminally tagged OVO had a persistent staining pattern from germline stem cells throughout developing egg chambers, while N-terminally tagged OVO-A and OVO-B showed a weaker staining intensity in the germline stem cells and region G1 of the germarium, stronger staining in region G2A, and then reduced in staining intensity in the remaining G2B and G3 regions of the germarium. This difference in staining intensities between the location of the tag within OVO, and preliminary western analysis raises the possibility of protein level regulation. We also looked at the expression of OVO in the adult male germline and were not able to detect the presence tagged OVO-A, but we were able to detect weak expression of N- and C-terminally tagged OVO-B in spermatogonia, but not spermatocytes, spermatids, or sperm. OVO's expression and localization during embryogenesis is unknown so we decided to determine its maternal and zygotic activity throughout embryonic development. We found that OVO is maternally loaded into the embryo, where it showed no overt localization until right before cellularization, when it became nuclear in the newly formed pole cells. Maternal OVO persisted in the migrating germ cells. In newly formed embryonic gonads (stage 15 of embryogenesis) maternal OVO still persists and zygotic OVO expression is detectable, indicating that there is continuous nuclear OVO expression in the female germline.

401F **Characterizing the function of dSLC25A46b, a mitochondrial shaping protein, in *Drosophila* spermatogenesis and in the nervous system** Claire Olson, Amelia Roselli, Sam Kavarana, Caroline Phan, Tommy Mason, Vivienne Fang, Karen G HalesDavidson College Department of Biology

During *Drosophila* spermatogenesis, mitochondria undergo significant structural changes, allowing the examination of genes that regulate mitochondrial shaping. The *Z2-3738* recessive mutant strain, originating from the Zuker collection, displays dysfunctional spermatogenesis, including male sterility and disorganized sperm bundles with scattered mitochondrial aggregates. The *Z2-3738* strain has a nonsense mutation in *dSLC25A46b (CG5755)*, a testis-specific paralog of a broadly expressed gene. Its human ortholog, SLC25A46, a modified solute carrier protein, functions in mitochondrial

fission and has been implicated in several human neurodegenerative diseases. Thus, elucidating the function of its *Drosophila* ortholog may provide insight into these conditions. Here, we characterize the function of *CG5755* by exploring its interactions with other mitochondrial proteins and its putative role in the nervous system as proposed by others. SLC25A46 has been hypothesized to interact with the mitochondrial membrane phospholipid cardiolipin. We investigate the interaction between CG5755 and cardiolipin by assessing localization of a *CG5755-GFP* transgenic protein under knockdown of cardiolipin synthase (CLS) and determining whether a synthetic phenotype exists under conditions of CG5755 mutation and CLS knockdown. Preliminary results indicate that knockdown of CLS alone can lead to male sterility and dysfunctional spermatogenesis. We also characterize interactions between CG5755 and the testis-specific ADP/ATP carrier protein Ant2*; Ant2* knockdown alone leads to male sterility. Additionally, although current RNA-seq data report *CG5755* as testis-specific, another group reported phenotypes in neuron-specific *CG5755* knockdown flies. We found a reduction in climbing ability in *CG5755* mutant female flies, but not male flies. Surprisingly, *Z2-3738/Df(2L)Exel7070* flies showed significantly faster climbing speed than *CG5755* homozygous mutants and wild type flies, suggesting a possible genetic interaction between CG5755 and a neighboring gene. We failed to observe significant expression of *CG5755-GFP* in nervous system tissues, but cannot rule out small areas of expression. We also report results of qPCR measuring expression of *CG5755* in larval and adult brain tissue. Together, these results provide preliminary evidence about the interaction of CG5755 with other mitochondrial proteins and attempt to reconcile conflicting data over its expression in the nervous system.

402F **Characterizing the composition and morphology of the germ plasm in the wasp *Nasonia vitripennis*** Allie Kemph[1], Jeremy Lynch[1], Alexey Arkov[2], Kabita Kharel[2], Samuel Tindell[21]Biological Sciences, University of Illinois at Chicago, [2]Biological Sciences, 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 that drives germ cell fate determination. There is great variability in morphology and composition among organisms that contain germ plasm. For example, in *Drosophila* the germ plasm consists of many relatively small granules that remain associated with the posterior pole of the egg until they are taken into individually budding poles cells. In contrast, in the wasp *Nasonia vitripennis,* germ plasm assembles into an extremely large, dynamic structure, called the “oosome”. The oosome migrates anteriorly to 50% egg length, before returning to the posterior pole where a single large bud containing multiple nuclei emerges preceding pole cell formation. While much is known about the composition of the fly germ plasm, how the structure of the oosome compares is as yet unknown. Here we describe our progress in characterizing the dynamic oosome morphology and determining the spatial arrangement of several oosome mRNA and protein components. Our initial results show that the oosome is a complex, non-homogeneous structure with high density mRNA puncta, lacunae apparently devoid of mRNA, and distinct domains of specific protein accumulation. We also find that the oosome shape changes dramatically during early embryogenesis, indicating that it has liquid or hydrogel-like physical properties.

403F **SIRT1 is required for cohesion maintenance during meiotic prophase in Drosophila oocytes** Zihan Meng[1], Nicholas G Norwitz[2], Sharon E Bickel[11]Biological Sciences, Dartmouth College, [2]Harvard Medical School

Meiotic chromosome segregation errors are the leading cause of miscarriages and aneuploid pregnancies and their incidence increases dramatically as a woman ages, a phenomenon called the maternal age effect. In meiotic cells, cohesive linkages between sister chromatids physically hold recombinant homologs together and accurate chromosome segregation in human oocytes requires that sister chromatid cohesion remain intact for decades. Age-dependent loss of meiotic cohesion during the long prophase arrest of human oocytes contributes to the increased incidence of segregation errors in older women. SIRT1 (also known as Sir2) is a NAD^+ dependent deacetylase that has been shown to suppress age-related pathologies in several cell types and organisms. To test the hypothesis that SIRT1 functions in oocytes to promote cohesion maintenance, we utilized a Gal4-UAS strategy to induce SIRT1 knockdown during meiotic prophase in Drosophila oocytes and assay the fidelity of chromosome segregation. Knockdown of SIRT1 using two different hairpins caused a significant increase in meiotic segregation errors, including those of recombinant homologs, consistent with premature loss of meiotic cohesion. We utilized FISH to directly visualize the state of cohesion in mature Drosophila oocytes and found that SIRT1 knockdown during prophase causes a significant increase in arm cohesion defects. Together, our results indicate that SIRT1 is required in prophase oocytes to ensure that meiotic cohesion is maintained. Our working model is that aging causes a decline in SIRT1 levels/activity that contributes to age-dependent segregation errors. We are currently pursuing experiments to test this hypothesis.

404F **Autophagy during meiotic prophase is required for accurate chromosome segregation in Drosophila oocytes** Diana C Hilpert, Sharon E BickelBiological Sciences, Dartmouth College

Aneuploid pregnancies and miscarriages increase dramatically during a women's thirties because meiotic segregation errors are much more prevalent in the oocytes of older women. This phenomenon, termed the maternal age effect, arises in part because meiotic sister-chromatid cohesion deteriorates during the decades long arrest that human oocytes spend in prophase I. One hallmark of aging is oxidative stress. Our laboratory has previously shown that induction of oxidative stress in Drosophila oocytes during meiotic prophase causes premature loss of cohesion and segregation defects. Furthermore, we have demonstrated that age-induced segregation errors in Drosophila oocytes are suppressed by modest overexpression of enzymes that scavenge reactive oxygen species (ROS). One cellular defense against oxidatively damaged molecules is autophagy, a process by which damaged organelles, protein aggregates and specific proteins are enveloped and degraded. By sequestering and destroying damaged mitochondria, a major source of intracellular ROS, autophagy is crucial for the maintenance of cellular ROS homeostasis. Several lines of evidence indicate that autophagy declines with age and that stimulation of autophagy protects aging cells from functional decline.

To ask whether accurate segregation in Drosophila oocytes depends on normal levels of autophagy during meiotic prophase, we used a Gal4/UAS inducible RNAi strategy to knock down different autophagy proteins and quantify segregation errors in knockdown versus control. We find that meiotic segregation errors are significantly elevated when Atg8a or Atg1 is knocked down during meiotic prophase. Knockdown also causes a significant increase in the missegregation of recombinant homologs, consistent with premature loss of sister chromatid cohesion. Moreover, we have confirmed that prophase knockdown of additional autophagy proteins that impact different steps in the macroautophagy pathway also disrupt meiotic chromosome segregation, supporting the model that basal levels of autophagy are required during meiotic prophase to promote accurate chromosome segregation. Experiments are currently underway to quantify autophagic foci in Drosophila oocytes in knockdown and control oocytes. Characterization of autophagy in prophase oocytes is essential to understand how this pathway promotes normal meiotic segregation and our studies may also inform clinical approaches to stimulate autophagy in aging oocytes.

405F **Defining the mechanistic basis of germline transcriptional activation** Anais Tsai[1], Sheri Grill[2], Ruth Lehmann[21]Biology, Massachusetts Institute of Technology, [2]Biology, Whitehead Institute

Initiation of transcription during the maternal to zygotic transition of embryos is distinct between germ cell and somatic cells in *Drosophila melanogaster* embryos. In somatic cells, zygotic activation of the genome occurs in two waves, a minor wave and a major wave. Zelda is a pioneer transcription factor that is necessary for zygotic transcription in both the minor and major wave of somatic transcription. Pioneer transcription factor binding of Zelda to TAGteam sites on the DNA permits the formation of nucleosome lacking regions that are then transcribed zygotically. Primordial germ cells maintain the inheritance of specialized germ cell determinants by excluding numerous maternally deposited transcripts. Maternal and early somatically transcribed Zelda RNA are part of the excluded transcript populations. Primordial germ cell zygotic activation occurs across more time with more waves than somatic transcriptional activation, however no transcription factor responsible for priming the germ cell genome has been previously identified. Surprisingly, our studies show Zelda protein is found in primordial germ cells. ATAC-Seq from primordial germ cells confirms transcription factor footprints at canonically Zelda TAGteam binding sites. Here we probe the function of Zelda protein in priming the DNA for initiation of the germ cell transcriptional program. Using a high-resolution variation of Fluorescent In-Situ Hybridization, Hybridization Chain Reaction FISH, in combination with different Zelda mutants, we are able to identify which portions of the germ cell transcriptional program rely on Zelda protein acting as a pioneer transcription factor.

406F **Test Requirement of Polo Kinase to CO Formation in Drosophila.** Bowen Man, Nicole CrownCase Western Reserve University

The Polo Kinase has been examined to be crucial in drosophila mitosis by serving a regulatory role. Previous research also suggested that S.cerevisiea and C.elegans Polo Kinase homologous are required for CO formation and progression through pachytene. However, the requirement and function of Polo Kinase in drosophila meiosis are unclear. In this proposal, I would practice cytologic analysis on Polo Kinase and crossovers in germarium with tagged Vilya protein to investigate the colocalization between Polo Kinase and crossover sites. I will also perform cytological analysis on hypomorphic Polo Kinase mutant to look for the potential role of Polo Kinase in Drosophila meiosis. The versatile function of Polo Kinase implies complex regulatory roles of this protein in multiple biological processes. By building the connection between Polo Kinase and crossover formation, we can expand our knowledge and toolbox in studying

meiosis.

407F **Regulation of stem cell niche assembly during gonadogenesis** Tynan Gardner[1], Stephen DiNardo[2], Lauren Anllo[31]Perelman School of Medicine at the University of Pennsylvania, [2]Cell and Developmental Biology, Perelman School of Medicine at the University of Pennsylvania, [3]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 niche. Unfortunately, we know little about how niches are compartmentalized within tissues during organogenesis. Using live-imaging techniques, we employ the developing testis as a model to study the genetic control of niche establishment. Before niche formation, the spherical gonad contains an interwoven mix of somatic cells encysting germ cells. Our imaging revealed that niche progenitor cells extend protrusions to pull themselves out to the gonad periphery, where they migrate along extracellular matrix toward the anterior. We found that the niche assembles with a tilt towards organs at the embryo interior, and that Slit and FGF signals emanating from adjacent internal visceral muscle (Vm) regulate niche assembly. In response to signaling, niche cells express the transcription factor *islet*, which we find is also required for positioning the niche (Anllo & DiNardo, 2022). Identifying regulators of *islet* expression would reveal if Vm signals Slit and FGF directly induce niche cell intrinsic signaling responses, or would identify novel key intermediaries involved in positioning the niche. To this end, we have shown that a minimal element from the *islet* enhancer region containing Org1 binding sites is sufficient to drive gene expression in niche cells. *org1* is a T-box transcription factor with known roles in specifying muscle development (Boukhatmi et al., 2014). Using a gene expression reporter and genetic mutants, we see that *org1* is both expressed in embryonic niche cells and required for their assembly at the gonad anterior. In the absence of *org1*, embryonic niche cells fail to express a key adhesion protein, Fas3, and remain dispersed throughout the gonad instead of forming a single organized cluster at the anterior. Further, *islet* expression is significantly reduced in the absence of *org1*. This work suggests that Org1 mediates *islet* expression in the embryonic niche. Future experiments will identify whether *org1* expression depends on known Vm signals, or will unveil additional signaling mechanisms that mediate compartmentalization of the testis niche during development.

408F **Regulation of Spermatogenesis by Notch and Ribbon** Allyson Terrell, Adrianna Soriano, Shannon McDonnell, Jennifer MierischLoyola University Chicago

Spermatogenesis is essential for production of sperm for sexual reproduction. Defects in this process can lead to infertility. In order to understand the basis of infertility it is necessary to identify and characterize the genetic mechanisms that promote sperm development. Previous studies involving *drosophila melanogaster* have demonstrated that Notch signaling is required in the somatic cells of the gonad to promote sperm development. We have shown that increased levels of Notch signaling in somatic cells results in the arrest of somatic cyst cell development in the testes and a subsequent arrest in spermatogenesis. In a parallel study we also observed that increased levels of the transcription factor Ribbon in somatic cells also arrests spermatogenesis. Given that testes with increased Notch and Ribbon exhibit similar phenotypes, we wanted to examine expression of potential target genes in the testes. Previous studies have demonstrated that the Drosophila homologue of mammalian Ras Responsive Element Binding Protein 1 (RREB1), known as *hindsight* (*hnt*), is a target of Notch signaling in other tissues, suggesting it may be a target in the testes as well. We find that Hnt is expressed in the somatic cells of the testes during the transition from early to late somatic cyst cells, when Notch signaling is active. When Notch is overexpressed, somatic cells persist in this transition state and Hnt expression is expanded. We also observed that Hnt expression is present in the early germline cells of the controls and expression of Hnt appears to be expanded in the germ line cells when Notch is overexpressed. Interestingly, when Ribbon is overexpressed, we observe less Hnt in somatic cells. These experiments suggest that Notch and Ribbon may not cooperate to promote spermatogenesis, but rather may act antagonistically. We are further exploring the relationship between Notch, Ribbon and Hnt to better understand how these genes, and their mammalian homologues promote spermatogenesis.

409F **Elucidating METTL3 Germline Function During *Drosophila* Spermatogenesis.** Rosemarie L Mirabella[1], Mary L Kuziak[1], Antonio L Rockwell[21]Biology, Susquehanna University, [2]Susquehanna University

Over the past several years, work on m^{6}A methylation has revealed its role in regulating aspects of RNA metabolism. The enzyme that adds the m^{6}A modification METTL3, is evolutionarily conserved and plays a crucial role in numerous biological processes such as gametogenesis. Gametogenesis regulation is crucial in ensuring the viability of future generations. In *Drosophila* testes, both somatic cyst cells and germline cells are key facets of spermatogenesis. Our previous work in *Drosophila* spermatogenesis provided evidence suggesting METTL3 regulates chic in somatic cyst cells.

However, to date no lab has proposed a mechanism for METTL3 in the germline of *Drosophila* testes. Our current work aims to determine the function of METTL3 in the germline. Germline specific knockdown using the GAL4/UAS system was used to investigate the function of METTL3. In the testes, METTL3 is localized in early-stage germline cyst. Data collected from immunostaining testes suggests germline knockdowns have decreased rates of spermatid individualization relative to sibling controls. Additionally, RT-qPCR analysis suggests that several genes required for normal spermiogenesis appear to be misregulated in germline knockdowns. The spermatid phenotype in conjunction with the misregulation of spermiogenesis genes likely corresponds to the issues with fertility we have observed. Germline specific knockdowns have decreased fertility over time compared to controls. Collectively, our data suggests a potential role for METTL3 in the germline. Depletion of METTL3 in early-stage germline cells appears to compromise late-stage germline cell development. Our findings may provide further insight into METTL3's role in metazoan reproduction.

410F **_Drosophila_ PhLP3 is Required for Spermiogenesis** Christopher M Petit[1], Claire Chaikin[2], Elizabeth Kojak[2], Michela Marra[2], Stefan M Kanzok[1], Jennifer Jemc Mierisch[11]Biology, Loyola University Chicago, [2]Loyola University Chicago

Phosducin-like protein 3 (PhLP3) has been shown to possess redox-activity and is believed to function as a co-chaperone in the folding of cytoskeletal proteins. The *Drosophila melanogaster* homolog of PhLP3 (dPhLP3) is encoded by the previously uncharacterized *CG4511* gene. We find that dPhLP3 plays a role in the regulation of spermiogenesis in *Drosophila melanogaster.* Males homozygous for a P-element inserted in the 5' UTR of *dPhLP3* exhibit decreased *dPhLP3* expression, infertility, and a failure to produce mature sperm. Further examination of these *dPhLP3*$^{-/-}$ 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, are reduced in size, suggesting a reduction in the number of MTs in the DC. Thus, nuclei may fail to fully elongate due to a defective DC. As a result, mature sperm are not produced in *dPhLP3* 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 dPhLP3 as a co-chaperone for cytoskeletal proteins, we hypothesize that dPhLP3 functions to regulate the microtubule dynamics that are vital for DC formation and the nuclear-shaping process during spermiogenesis. We are currently exploring this hypothesis. dPhLP3 is well-conserved in humans, suggesting that our results on dPhLP3 function may provide insight into the molecular basis of infertility across species.

411F **Characterization of the role of _Notch_ in _Drosophila_ testes** Christine E Severude[1], Adriana Soriano[1], Heather Wheeler[2], Jennifer Mierisch[11]Biology, Loyola University Chicago, [2]Bioinformatics, Loyola University Chicago

The process of gonad development and gametogenesis is crucial for the propagation of our species and the conservation of biodiversity. The gene Notch plays an important role in these processes and is widely conserved across animal phylogeny. In humans and mice, lack of Notch has been associated with spermatogenesis arrest, suggesting that the differentiation and survival of male germ cells is dependent on the Notch signaling pathway. However, how Notch functions in this process is not well understood. Previous studies in Drosophila have shown that a loss of Notch signaling in the embryo results in defects in hub establishment, indicating that Notch functions in gonad development. Others have demonstrated that Notch is required in somatic cells for germ cell survival. Recent work in our lab demonstrates that increased Notch signaling in somatic cells of the testes also negatively impacts spermatogenesis, resulting in spermatogenesis arrest and infertility. Despite these important roles, no transcriptional targets of Notch have been identified in the testes. The goal of this experiment is to determine the transcriptional targets of Notch in the testes through RNA-sequencing analysis, bioinformatics, immunohistochemistry, and in situ hybridization. These target genes will help us understand the process and regulation of spermatogenesis in Drosophila and across animal phyla. It will also pave the way for future research endeavors exploring the function of identified target genes. These genes could represent therapeutic targets for individuals dealing with infertility.

412F **The Interchromosomal Effect Promotes Crossovers on the Recombinantly Dormant 4th Chromosome in _Drosophila melanogaster_** Joseph Terry[1], Anjelyna Siamphone[2], Savana Hadjipanteli[2], Erica Berent[2], Nicole Crown[11]Biology, Case Western Reserve University, [2]Case Western Reserve University

The initiation of meiosis entails a series of uniformly distributed DNA-double stranded breaks (DSBs) across the genome. As meiosis progresses, DSBs face two repair outcomes, either yielding a crossover (CO) or non-crossover (NCO). Unlike DSB distribution, genome-wide CO distribution does not reflect such uniformity. In fact, only a fraction of DSBs are repaired as COs, while the majority of remaining DSBs are repaired as NCOs.

Whether a DSB is preferentially repaired as a CO or NCO is largely dependent on the interplay between CO-suppressing

and CO-promoting forces, the culmination of which is termed, “crossover patterning”. Disturbances in CO patterning have been linked to deleterious outcomes on both the individual, and the population level by increasing aneuploidy susceptibility, or, more broadly, decreasing genetic diversity across populations over time.

Through the lens of CO patterning, *D. melanogaster* females offer an intriguing case study. Although the first three chromosomes display hallmark signs of CO patterning, the 4th chromosome never undergoes meiotic crossing over in normal conditions—-the absence of COs is absolute. Two separate, but not mutually exclusive, hypotheses drive the rationale behind the absence of COs on the 4th: chromosome size and dense heterochromatin composition.

Here we highlight a novel approach to promote CO formation on the recombinantly dormant 4th chromosome. This is accomplished by introducing a heterozygous balancer chromosome, subsequently triggering the interchromosomal (IC) effect. During the IC effect, unbalanced chromosomes recombine at increased frequencies, contrary to the balanced pair, in regions where COs are suppressed. By inducing the IC effect with four different balancer chromosomes, independently, we observe COs on the 4th at an average frequency of 1.2 cM between markers ci^{1} and ey^{R}. Further, to rule out the possibility of marker reversion, we present data of COs on the 4th between two different *attP* landing sites marked with RFP and y^{+}. Lastly, we will present data to determine if these COs are governed by CO patterning, by evaluating their dependence on meiotic resolvase mei-9. Because COs can also occur on chromosome 4 in the absence of blm, we propose a model where crossing over on chromosome 4 is generally dormant unless CO patterning mechanisms have been lost.

413S **Transcriptional and mutational signatures of the Drosophila aging germline** Evan Witt, Christopher B Langer, Nicolas Svetec, Li ZhaoThe Rockefeller University

In many species, including humans, older fathers pass on more paternally-derived *de novo* mutations. However, the cellular basis and cell types driving this pattern are still unclear. It is also unknown whether there is a hotspot of de novo mutation in the germ cell stages or not. To study the root causes of this phenomenon, we performed single-cell RNA-sequencing (scRNA-seq) on testes from young and old male *Drosophila,* as well as genomic sequencing (DNA-seq) on somatic tissues from the same flies. We found that early germ cells from old and young flies enter spermatogenesis with similar mutational loads, but older flies are less able to remove mutations during spermatogenesis. Mutations in old cells may also increase during spermatogenesis. Our data reveal that old and young flies have distinct mutational biases. Many classes of genes show increased post-meiotic expression in the germlines of older flies. Late spermatogenesis-biased genes have higher dN/dS than early spermatogenesis-biased genes, supporting the hypothesis that late spermatogenesis is a source of evolutionary innovation. Surprisingly, genes biased in young germ cells show higher dN/dS than genes biased in old germ cells. Our results provide novel insights into the role of the germline in *de novo* mutation.

414S **Determining how Doublesex and sex-specific steroid hormone signaling control gonad development** Samantha C Goetting[1], José Pac Cordero[2], Mark Van Doren[21]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), the founding member of the conserved Doublesex/Mab-3 Related Transcription Factor (DMRT) family. 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 interactions of BTB proteins. 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 in early gonad development: *mamo (maternal gene required for meiosis), chinmo (chronically inappropriate morphogenesis)* and *broad (br)*, a downstream effector of E signaling.

Loss of *mamo* in the somatic gonad leads to severely disorganized ovaries and loss of egg production. Conversely, loss of *chinmo* leads to formation of follicle-like cells in the testis. Upon examining *mamo* and *chinmo* RNA expression during development, I found that *mamo* is female-specific while *chinmo* is male-specific. Previous studies revealed a relationship between *chinmo* and *mamo* in the central nervous system: E signaling induces *mamo* and represses *chinmo*. *br* is a known E signaling target, and its knockdown in the somatic gonad causes severe ovary defects from L3 onwards. Given these observations, I hypothesize that *mamo* is critical for proper ovary development, and that it functions downstream of Dsx and E signaling (perhaps through the action of *br*) to help maintain a female sexual fate. I plan to characterize what cell types are affected in the absence of *mamo* and *br* using cell-specific markers and examine

how altering *dsx* and E signaling affect *mamo* and *chinmo* expression. 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.

415S ***bruno'*s role in dysgenic sterility : cellular responder or regulator of transposition?** Modupeola Bolaji, Erin KelleherBiology & Biochemistry, University of Houston

Transposable elements (TEs) are mobile genetic parasites whose mobilization leads to DNA damage and genomic instability. In *Drosophila melanogaster*, DNA damage resulting from the mobilization of *P*-element DNA transposons leads to a sterility syndrome known as hybrid dysgenesis. Dysgenic sterility occurs in the F1 offspring of crosses between P males (contain genomic *P*-elements) and M-strain females (lack both *P*-elements and *P*-element derived piRNAs that regulate *P*-element transposition). The absence of maternally deposited *P*-element derived piRNAs in the M strain eggs results in unrestricted transposition of paternally inherited *P*-elements in the F1 germline, triggering the loss of larval primordial germ cells (PGCs) and adult germline stem cells (GSCs).

Our lab recently identified *bruno* as a likely source of natural variation in *P*-element induced female dysgenic sterility. Furthermore, *bruno* loss of function alleles act as strong suppressors of *P*-element-induced dysgenic sterility. Bruno is an RNA-binding protein and a translational repressor that plays important roles in the oocyte development in the adult stage, including germ cell differentiation and cell cycle regulation. However, *bruno* is not known to regulate *P*-element transposition or to determine cellular responses to *P*-element transposition. Furthermore, while dysgenic germ cell loss starts in larval stages, *bruno* has no known function in larval germline.

To determine if *bruno* impacts dysgenic sterility in larvae or adults, we examined if *bruno* function impacts *P*-element induced PGC loss, GSC loss, or both. We discovered that Bruno function impacts *P*-element induced germ cell loss specifically in the larval PGCs, and also that Bruno protein is highly and specifically expressed in larval PGCs. We are currently testing two hypotheses to explain the relationship between *bruno* function and dysgenic PGC loss: 1) *P*-element transposition induces *bruno* expression or activity in PGCs, resulting in PGC loss through *bruno*-dependent promiscuous differentiation or reduced proliferation and 2) *bruno* facilitates transposition of *P*-element in PGCs, leading to DNA damage and loss of PGCs. This study will reveal a novel function of *bruno* in PGCs, as well as a novel type of interaction between *P*-elements and their hosts.

416S **Characterizing the Role of Doublesex in Creating Sexual Dimorphism in the Somatic Gonad** Natalie A Murphy, Ellen Baxter, Mark Van DorenBiology, Johns Hopkins University

Doublesex (Dsx) and Mab-3 Related Transcription factors (DMRTs) are paramount for sex determination across the animal kingdom. In flies, the embryonic gonad is formed when a bipotent cluster of somatic gonadal precursors (SGPs) coalesces with germ cells. 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. Dsx, like mammalian Dmrt1, is first expressed in the somatic gonad during embryogenesis and is required for male vs. female gonad development. It is known that *dsx* is expressed in early SGPs during development, but the exact timing and role of Dsx in sex-specific cell fate specification during gonad development is unknown. We are interested in understanding when and where Dsx acts to control gonad sexual dimorphism.

We created an endogenously tagged GFP-Dsx to characterize Dsx expression in the somatic gonad. In both sexes, Dsx expression is initially promiscuous and seen in nearly all somatic cell types. It restricts by adulthood, and this restriction is temporally sexually dimorphic. In adult females, Dsx is limited to cap cells and anterior escort cells, and is clearly absent in the terminal filaments (TFs), follicle stem cells (FSCs), and follicle cells. In adult males, Dsx is expressed in the hub, cyst stem cells, early cyst cells, and terminal epithelium. Interestingly, the male niche and somatic stem cell population both retain their Dsx expression into adulthood, whereas TFs and FSCs in females lose Dsx. Additionally, in L3 and adult males, we observed combinatorial colocalization of Dsx with other known cyst cell markers (Zfh1, Tj, and/or Eya). We hypothesize that the order of expression of these molecular markers indicate the different stages of cyst cell differentiation.

Overall, these data point towards a more global need for Dsx in the soma during development, and a more limited need

for “maintenance” for adult gonad homeostasis. We hypothesize that in XY gonads, the role of Dsx^M in maintaining the established male identity is first seen in larval stages; while in XX gonads, which are largely quiescent during embryogenesis, the role of Dsx^F in maintaining the established female identity takes place during larval and pupal stages. We are making clones of 1) *dsx* LOF and 2) sex-switched *dsx* isoforms to further investigate this hypothesis and understand which cells of the gonad require autonomous sex information.

417S **E93 an Adult Transformer** Warda R Merchant, Cecilia Yip, Young You, Joel ElmquistInternal Medicine: Center of Hypothalamic Research, University of Texas Southwestern Medical Center

E93 (aka Eip93F) is required for adult development by regulating expression of larval genes during metamorphosis. In addition, we show that neuronal E93 regulates adult metabolism and biological rhythm (https://www.biorxiv.org/content/10.1101/2022.10.06.511196v2). When we knocked down E93 in neurons using a neuron-specific GAL4 (nSyb-GAL4) and UAS driven E93 RNA inference (nSyb>E93RNAi), the progeny became obese with increased attraction to food and disrupted circadian rhythm. Based on the known roles of E93 in termination of neuroblasts in the mushroom body during metamorphosis and our observation that neuronal knockdown of E93 shows increased attraction to food as if they were larvae, we hypothesize that neuronal E93 regulates neural re-wiring necessary for adult behavior. To test this hypothesis, we first examined whether nSyb>E93RNAi flies could mate. When we set up a fertility assay where an individual male and an individual female were allowed to mate for 24 h, we found that nSyb>E93RNAi males failed to mate. Because nSyb>E93RNAi flies also eclose with shriveled wings and because wings contribute significantly to courtship behavior, nSyb>E93RNAi males’ unsuccessful reproduction could be due to failure in attracting mates because they could not perform the wing dance, or because they were prioritizing feeding over courtship as if they were larvae. To investigate the first possibility, we set up our second fertility assay and tested nSyb>E93RNAi males with shriveled wings against control males with their wings clipped off. The results suggested that control males with their wings removed were still able to reproduce successfully while nSyb>E93RNAi males with shriveled wings did not. Currently we are investigating whether the nSyb>E93RNAi males prefer feeding over courtship based on the study from Lin et al (https://pubmed.ncbi.nlm.nih.gov/35140404/) where nutrients could determine a switch between courtship and feeding.

418S **The Creation of a Null Allele of *Clipper* and Investigation of Its Role in *Drosophila melanogaster* Oogenesis** Charlie T Watts, Julie A MerkleBiology, University of Evansville

The process by which the oocyte identity is determined in the *Drosophila* ovary is not well understood. An EMS mutagenesis screen was performed on 2L to better understand the molecular mechanisms that control this process, and an allele of *Clipper (Clp)* was identified. *Clp* germline clones exhibit a phenotype consisting of 16 nurse cells, no oocyte, and a clustering of ring canals within the mutant egg chamber. *Clp* encodes a protein component of the Cleavage and Polyadenylation Specificity Factor (CPSF) complex that recognizes the AAUAAA signal sequence at the 3’ end of primary transcripts and recruits poly(A) polymerase and other cleavage and polyadenylation factors. Polyadenylation is important for mRNA stability, export from the nucleus, and recognition by the ribosome. To investigate how a genetic null of *Clp* affects oogenesis, CRISPR/Cas gene editing was used to generate a knock-out allele of *Clp*. A gRNA plasmid was generated using Gibson Assembly to assemble two gRNAs into the pCDF5-w backbone, while a homology domain plasmid was created by ligating homology arms that flank the *Clp* genomic region into pHD-EGFP-attP. These plasmids were then injected into a germline-expressing Cas9 line. Transformants were screened for GFP, and two potential lines were identified. These lines will be validated by complementation analysis and genomic sequencing, and then characterized by phenotypic analysis compared to the EMS-generated line and FRT controls. The ultimate goal of this project is to better understand the role of *Clp* in oogenesis, thereby providing insight into the molecular mechanisms of oocyte identity.

419S **A meiosis specific APC/C functions in sex determination** Osamah Batiha[1], Abuzar Sikandar[2], Rachel Andrews[2], Eric Fifield[2], Rami Mechael[2], Andrew Swan[21]Jordan University of Science and Technology, [2]Biomedical Sciences, University of Windsor

Cort (Cortex) is a meiosis-specific activator of the Anaphase Promoting Complex/Cyclosome (APC/C) that is essential for the completion of female meiosis and for several events in the transition of egg to embryo. Cort expression is tightly regulated - it is transcribed only in the oocyte, and the protein appears to be rapidly cleared from the embryo following meiosis. To determine the significance of this tight regulation, we mis-expressed Cort throughout development. Surprisingly, the zygotic mis-expression of Cort leads to a transformation of females to a male-like

fate. This transformation depends on Cort interaction with the Anaphase Promoting Complex/Cyclosome (APC/C), suggesting that it involves the E3 Ubiquitin ligase activity of APC/C^{Cort}. Genetic and molecular evidence indicates that Cort targets the splice factor Tra for destruction. Using a conditional allele combination of *cort* that partially rescues its meiotic requirement, we find that loss of *cort* in the female germline disrupts the sexual identity of male progeny due to inappropriate initiation of the female sex determination pathway in these males. Surprisingly, maternal Cort does not affect sex determination through Tra. Rather, our evidence to date suggests that maternal APC/C^{Cort} targets one or more of the transcription factors that activate early Sxl transcription in female embryos. Thus, maternal Cort functions to prevent the inappropriate initiation of the Sxl positive feedback loop in male embryos. While Sxl is the key upstream determinant of sex in Drosophila, in most insects, Tra has this role. We hypothesize that the ability of Cort to target Tra in Drosophila reflects an ancestral role for APC/C^{Cort} in targeting maternal Tra to prevent the inappropriate initiation of a Tra positive feedback loop that establishes female fate. Thus APC/C^{Cort} may play a conserved role in erasing the female identity of the egg to allow the chromosome-based sex determination pathway to determine the sex of the individual.

420S **Mutations in RNA Helicase Me31B's key motifs influence germ cell quantity in *Drosophila*** Megan Proffer, Evan Kara, Ming GaoIndiana University Northwest

Me31B is an ATP-dependent DEAD-box RNA helicase abundantly found in *Drosophila* germline. It forms Ribonucleoprotein (RNP) complexed with other germline proteins such as Tral, Exu, and Cup to silence mRNA transcripts until they have localized to the correct location of developing eggs, a process critical for proper germ cell formation. However, the molecular mechanism of how Me31B contribute to germ cell formation is not well understood. To study this, we used CRISPR technique to generate *Drosophila* strains carrying genes mutant for key functional motifs: DEAD-box motif, DVLARAK motif, N-terminal domain, C-terminal domain, and QAHR motif. To analyze the germ cells formed in the mutants, we collected stage 10-11 and NC 14 embryos from the mutant strains, stained the embryos with anti-Vasa antibodies to mark the germ cells, and quantified the germ cells. From our analysis, we report germ cell phenotypes such as germ cell numbers reduction and germ cells undergoing premature apoptosis in certain strains. These germ cell phenotypes from different *me31B* mutants gave us further insight into how Me31B protein (and its motifs) participates and regulates *Drosophila* germ cell formation and development.

421S **Spargel/dPGC-1 RNA Recognition Motif (RRM) is essential for Oogenesis** Swagota Roy[1], Sabarish Nagarajan[2], Atanu Duttaroy[31]Biology, The Howard University, [2]Genetics/ Biotechnology, Morgribge institute for research/ University of Wisconsin, Madison, [3]Biology, Howard University

Spargel (srl), is the single Drosophila homolog of mammalian transcription co-activator PGC-1. Spargel offers some unique opportunity to explore the ancestral function(s) of PGC-1 gene family particularly why it remains conserved in mammals. Ovary-specific knockdown of *srl* stops egg chamber development resulting in rudimentary ovaries which fails. to produce any eggs. Therefore, Spargel plays an essential role in ovarian growth and ultimately to female fertility. The three vertebrate homologs of PGC-1 and its invertebrate ortholog Spargel/dPGC-1 all carry an RNA recognition motif (RRM) and a RS domain in their C-terminal ends. Although *in vitro* studies predicted RNA processing capacity of PGC-1's RRM domain, *in vivo* analysis with a splice variant NT-PGC-1a, that is lacking the RRM and the RS domains fail to support hallmark functions of PGC-1s, maybe because of functional redundancy between the homologs. Structural comparison between Drosophila Spargel and mammalian PGC-1 RRM and RS domains revealed significant homology (68% positive residues) between these two domains. Therefore, Spargel can be an excellent tool to reassess the role of RRM and RS domain *in vivo*. To further assess the involvement of RRM domain on oogenesis we deleted the endogenous RRM domain of *srl* (DRRM). This domain-specific *srlΔRRM* mutants lays nearly no eggs since their ovaries grow slowly leading to a massive accumulation of advanced stage egg chambers but no mature eggs. *srlΔRRM* stage 9 and above egg chambers displayed weak, partial, or missing cortical actin and actin cable formation which explains the dumpless phenotype of the *srlΔRRM* egg chambers since nurse cell contents cannot be dumped to the oocyte due to actin cables malformation On the contrary when *srlΔRRM* is overexpressed in a wild type background ovaries are severely underdeveloped because of excess cell death. This condition is even deteriorated when a combined SrlΔRRM+ΔRS protein is overexpressed. This suggests that truncated Spargel protein is overshadowing wild Spargel activity resulting in a dominant negative effect. Altogether our findings highlight the specific requirement of RRM domain of Spargel in *Drosophila* oogenesis.

422S **Establishing *Drosophila* egg chambers as a cell size control model** Shruthi Balachandra, Amanda A AmodeoBiological Sciences, Dartmouth College

Cells must maintain a characteristic size necessary to perform their physiological functions. In most cell types they grow to a given volume before dividing. However, some unusually large cells such as the growing *Drosophila* oocyte use different size control strategies. The egg chambers that give rise to the oocyte consist of an oocyte, its neighboring 15 nurse cells, and a signal layer of follicle cells which provide yolk and structural support to the growing egg chamber. These 15 nurse cells uncouple cell division and enter the endocycle to synthesize the necessary maternal molecules. Once they reach their maximum sizes they dump all their cytoplasmic content into the oocyte. This defined number of endocycling nurse cells offers a simple model to understand cell size decisions in a situation where a major developmental change is dependent on achieving the correct size. On the other hand, the oocyte which receives all the content from nurse cells, and also receives yolk from follicle cells attains a critical size with the right concentration of molecular regulators to support early embryonic cell cycles. This chain of events following the maximum size attended by the nurse cells suggest a collective role of all these cell types in making their own size decisions and the organ size, that is the size of the egg chamber. To address the cell control decisions during oogenesis we adapted Bellymount live imaging method as an alternative to short-term ovary cultures. Our preliminary observation with Bellymount provides a glimpse of real-time in vivo growth dynamics of the egg chambers, which is not feasible with other available imaging tools. Currently, we are manipulating the biosynthetic capacity of the nurse cells and the cell-cell interaction between nurse cells and follicle cells to uncover the molecular mechanism underlying cell size regulations during *Drosophila* egg development.

423S **Identifying the Protein Factors That Influence the Aggregation of Me31B into *Drosophila melanogaster* Germ Granules** Ammaar R Mohammed, Deep Govani, Ming GaoIndiana University Northwest

Me31B (DDX6), a DEAD-box RNA helicase, is an important component in the germ granule RNPs (ribonucleoproteins) that are crucial for germline development in *Drosophila* melanogaster. Our previous research showcased several *me31B* mutant strains in which Me31B fail to aggregate into germ granules *in vivo*. For instance, the $me31B^{N\text{-}ter}$ and $me31B^{FDF}$ strains exhibited dispersed Me31B protein distribution in the nurse cells and oocytes. In this study, we aim to understand the molecular mechanism of the mutant Me31B proteins' failure to aggregate. Previous research suggests that Me31B interacts with other germline proteins in the germ granules, a process that may recruit Me31B into granule RNPs. We therefore hypothesize that Me31B's *in vivo* interacting partners provide the necessary support for Me31B's proper aggregation. Therefore, we conducted co-immunoprecipitation (Co-IP), SDS-PAGE, and mass spectrometry against the wildtype and mutant Me31B proteins to determine the discrepancies in their interacting partners. We successfully identified several protein candidates that differentially interact with the wildtype vs the mutant Me31B. We reason that these protein factors could influence Me31B's aggregation statues in the germline. Our study provides further insights into the assembly mechanism of Me31B-containing germ granule RNPs.

424S **Understanding rare disease variants of the conserved nuclear lamina protein Barrier-to-Autointegration Factor** Samuel D Jones, Dainel X Haws, Pamela K GeyerBiochemistry, University of Iowa

Barrier-to-autointegration factor (BAF/BANF) is a conserved nuclear lamina (NL) protein essential for nuclear integrity, chromatin structure, and genome stability. Whereas complete loss of BAF causes lethality, extremely rare cases of human disease have been identified that result from expression of BAF variants. One variant, Ala12Thr, was identified in three unrelated individuals who shared a recessive premature aging syndrome. The second variant, Gly16Arg, was identified in a single individual who had a dominant progressive neuromuscular disease. Disparate disease phenotypes suggest that mutations affect different functions of BAF. The Human and fly BAF share 65% amino acid identity including identity of the two mutated residues, therefore we studied the functions of these two variants in the fly. We first used CRISPR lines to study recessive phenotypes associated with the Ala12Thr variant and GAL4-UASp responder lines to study dominant phenotypes of the Gly16Arg variant. Unlike complete loss of BAF, adults expressing either variant survive, demonstrating that disease variants retain some of BAF functions. To extend our understanding, we have focused our investigations on oogenesis, as BAF is required for germline stem cell (GSC) survival and continuous oocyte production. We find that both BAF variants affect oogenesis, with evidence that GSC mitosis is altered. Notably, we link mitotic dysfunction of the Ala12Thr variant to decreased recruitment of centromeric proteins of the kinetochore during GSC division, indicating that the Ala12Thr mutation affects production of a dephosphorylated, localized pool of BAF required for centromere assembly and accurate chromosome segregation. Preliminary studies of the Gly16Arg mutants suggest that GSC mitosis is also affected, but through a different mechanism. Taken together, these studies are advancing our understanding of how disruptions in BAF function interfere with tissue homeostasis, leading to disease.

425S **Slogging through Mud: meiotic isoforms and their function** Nick Lowe, Tara Finegan, Kevin Deem, Daniel

BergstralhUniversity of Rochester

Meiosis is ubiquitous among multicellular organisms and key to sexual reproduction. Previous studies implicate Mud in several oocyte processes – these include synapsis, nuclear migration, and spindle cohesion at meiosis II – but how Mud participates is incompletely understood. Mud is well-established as the fly homolog of two proteins, vertebrate NuMA and nematode Lin-5, that are studied as regulators of microtubule organization. However, at the sequence level conservation between these proteins is low. Only a relatively short stretch, called the NLM sequence, is very similar between Mud homologs, and it can be detected across a long evolutionary span. This sequence is thought to include both a microtubule-binding domain and the binding site for an important partner protein called Pins/LGN/GPR1-2 (flies, vertebrates, worms) that helps Mud to drive mitotic spindle orientation. Remarkably, flies encode isoforms that do not include the NLM. This raises a question: is the only well-conserved region dispensable for some functions? Our work suggests that the isoforms lacking the NLM participate in meiosis. We show that these isoforms are expressed in the testis and ovary but not in the somatic tissues we examined. Additionally, whereas Mud has established roles in the oocyte, we show here that it is also at the nuclear envelope of developing sperm. We are now investigating the question of how isoforms lacking the NLM work.

426S **Does 4-nonylphenol affect the fertility of Drosophila?** Jennifer BanduraLock Haven University

Endocrine disrupting chemicals (EDCs) are commonly found dispersed in the environment and may pose a threat to human health. These chemicals come from many everyday products, including plastic containers and detergents, and some can persist for long periods of time in soil and water. EDCs interfere with the biosynthesis, metabolism, or action of hormones, and they can negatively affect reproduction. We have focused specifically on the effects of 4-nonylphenol, an EDC that is particularly prevalent in the environment. 4-nonylphenol has been shown to have a definitive feminizing effect on fish. However, based on published literature, the impact of 4-nonylphenol on the fertility of *Drosophila* is currently not clear. To determine if 4-nonylphenol influences the fertility of either male or female *Drosophila*, we will compare the number of eggs laid and rates of egg hatching between parental flies exposed to 4-nonlyphenol and control flies.

427S **Essential and recurrent roles for endogenous RNAi to silence *de novo* sex chromosome conflict**
Jeffrey Vedanayagam[1], Marion Herbette[2], Holly Mudgett[3], Ching-Jung Lin[1], Caitlin McDonough-Goldstein[4], Stephen Dorus[4], Benjamin Loppin[2], Colin Meiklejohn[3], Raphaelle Dubruille[2], Eric Lai[11]Sloan Kettering Institute, [2]Université de Lyon, [3]University of Nebraska, [4]Syracuse University

Meiotic drive loci distort the normally equal segregation of alleles, which can benefit their own transmission even in the face of severe fitness costs to their host organism. However, relatively little is known about the molecular identity of meiotic drivers, their strategies of action, and mechanisms that can suppress their activity. Here, we present data using the fruitfly *Drosophila simulans* that address these questions. We show that a family of *de novo*, selfish, protamine-derived X-linked factors (the *Dox* gene family) is silenced by a pair of newly-emerged hairpin RNA (hpRNA) siRNA-class loci, Nmy and Tmy. Knockout of *nmy* causes loss of males, due to derepression of *Dox* and *MDox*, whereas knockout of *tmy* renders males sterile due to misexpression of *PDox* genes. Notably, genetic interactions between *nmy* and *tmy* mutants indicate that Tmy also restricts sex ratio bias. Moreover, PDox protein localizes to chromatin bridges in affected meiotic figures of *tmy* mutants, indicating a chromosomal function. Finally, we show these target loci are polymorphic within *D. simulans*, and strikingly, that both hpRNA mutants can be rescued by wild X chromosomes bearing natural deletions in different Dox family genes. Altogether, these studies support a model in which repeated cycles of sex chromosome conflict and resolution can drive genome evolution, remodel gametogenesis, and mediate reproductive isolation.

428S **ESCRTs mediate Notch signaling in the testis stem cell niche** Mara R. Grace, Erika MatunisCell Biology, Johns Hopkins University

Stem cell niches are dynamic microenvironments that provides 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. While 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, specifically that of 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 area, thus suggesting that ESCRTs mediate signaling from somatic stem cells back to their niche to prevent niche hypertrophy. 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 while expression of activated Notch in somatic stem cells results in an enlarged niche, thus suggesting that Notch signaling mediates the observed niche hypertrophy caused by ESCRT loss. This leads to a model where loss of ESCRTs in somatic stem cells leads to an autononomous 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.

429S **Uncovering the Mechanisms that Activate the Germ Cell Transcriptional Program** Sherilyn Grill[1], Inma Barrasa[1], Anais Tsai[1], Monica Selvaraj[2], Ruth Lehmann[1]1Whitehead Institute for Biomedical Research, [2]Department of Cell & Developmental Biology, Weill Cornell Medicine

Germ cells contain the extraordinary potential to generate every cell in the body. This totipotent potential is established in the germline at the earliest stages of development and must be maintained for the duration of an individual's reproductive lifespan, a task that can last more than forty years in humans. To overcome this challenge, germline regulators activate the germline transcriptional program while simultaneously protecting germ cells from reprogramming to a somatic cell fate. In the dynamic embryo, a specific program for germ cell transcriptional activation has yet to be described, largely because a 'master-regulator transcription factor' for germ cell fate has not been identified. Here we use a multipronged approach to systematically characterize the primordial germ cell (PGC) gene expression program and identify prime regulators of germ cell fate. Using single cell RNA-seq analysis of PGCs isolated from Drosophila embryos, we identified six distinct transcriptional programs that occur throughout PGC development. Interestingly, two of these PGC transcriptional programs include transient, zygotically transcribed RNAs that are known to be expressed early in the embryonic soma. We have identified the pioneer factor Zelda as a potential activator of this transcription and are currently probing how loss of Zelda in PGCs impacts germ cell development. Using mutants that disrupt somatic tissues but not germ cell formation, we are asking if these PGC transcriptional programs are germ cell autonomous or impacted by signals from the surrounding somatic cells. To identify transcription factors that activate each of these programs, we performed ATAC-seq with footprinting analysis on isolated PGCs. In addition to known transcription factors, we have identified novel cis-acting sequences that are enriched in the open chromatin of PGCs and may regulate these germ cell transcriptional programs. We are currently working to identify trans-acting factors that interact with these novel sequences and uncover if their activity is sufficient to activate germline transcription. Together, our analyses have generated a quantitative view of the transcriptional transitions that occur during PGC development and begun to uncover the specific transcription factors that activate the germline genetic program.

430S **Using FIB-SEM and AT-SEM to monitor ultrastructural changes in the germline ring canals** Stephanie Pellegrino[1], Abigayle Elsbury[1], Irina Kolotuev[2], Lindsay Lewellyn[1]1Butler University, [2]University of Lausanne

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, size, or stability, but a more extensive analysis of ultrastructural changes is lacking. Electron microscopy is a valuable experimental tool that can be utilized to monitor structures through development or following genetic or other manipulations. Multiple studies have utilized Transmission Electron Microscopy (TEM) to monitor changes in ring canal structure, but due to the significant investment of time and resources, its use has been fairly limited. 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 several early-stage germ cell clusters. Although this analysis revealed interesting structural differences in the germline ring canals within a single cluster as well as through development, 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.

431S **Characterization of testis specific sugar transport and glycolysis genes in *Drosophila melanogaster*** Mark Hiller, Nicole Tolson, Lexie Alvarado, Julia GazzolaGoucher College

Sugar metabolism is required to build and maintain cellular structures and provide energy for cells. Regulation of cellular energy levels is in part dependent on the transport of sugars or other small molecules such as lactate. The *Drosophila melanogaster* genome contains twenty-five genes annotated as encoding SLC2 (solute carrier family 2) proteins that are believed to transport sugars, and five show testis-specific expression. During spermatogenesis, germline cells develop into sperm while surrounded by two somatic cyst cells. It is not clear how sugar molecules transit this barrier into germline cells. Germline cells also express testis-specific versions of several genes encoding glycolysis enzymes, but it is not known if they function during development or are necessary for function of mature sperm. We are using RNAi to assess the role of sugar transporters and glycolysis in germline and somatic cells during spermatogenesis.

432S **A single-nucleus gene expression atlas of the somatic female reproductive tract** Rachel C Thayer, Elizabeth Polston, David BegunEcology & Evolution, University of California, Davis

In addition to the ovaries, the Drosophila female reproductive tract includes a set of somatic tissues: the seminal receptacle, paired spermathecae, paired accessory glands, reproductive-associated fat bodies, oviduct, and bursa. These somatic tissues play crucial roles in sperm storage, fertilization, oviposition, and the female post-mating response. Moreover, they may be involved in sexual coevolution or sexual conflict. For example, seminal receptacle morphology and sperm morphology are strongly correlated across species, and female-secreted products influence sperm plug formation and ejection timing, which may have opposing fitness effects for females and males. Yet despite their functional importance and potential involvement in sexual coevolution, these somatic tissues have been understudied relative to the ovaries and male reproductive tissues. For example, these tissues were not targeted or annotated in the recent Fly Cell Atlas consortium, only the spermatheca is represented in FlyAtlas 2, and GAL4 drivers and other functional tools are lacking. Consequently, major questions about the function and evolution stand unsolved. Here, we present a single nucleus gene expression atlas of the six somatic female reproductive tissues, with cell types verified using in situ hybridization. We report previously unannotated cell types, including spatially distinct regions within the seminal receptacle. We also explore functional gene enrichments within cell types and cell-type specific signatures of evolution. Our data include wild-derived genotypes from high and low latitudes in the North American cline, allowing us to report evolutionarily conserved marker genes for each cell type. These data provide a resource toward developing cell-type specific GAL4 drivers and determining the distinct functional contributions of cell types within the somatic reproductive tract.

433S **Investigating the role of the microbiome in gonadogenesis** Taylar J Mouton, Nichole BroderickBiology, Johns Hopkins University

Many organisms, including humans, have microbes that live in their guts. In recent years, these microbes have been found to impact different aspects of host physiology such as immunity, gut repair, nutrition, and metabolism. Therefore, an organism's gut microbiome and its function have extensive impacts on organismal physiology and health. However, these features are often also influenced by environmental factors, most notably diet. Studies using Drosophila melanogaster and its microbiome have increased our understanding of such influences due to its relative low-complexity and the ability to stably culture the resident bacteria in the laboratory. Interestingly, more recent studies have found that the parental microbiome status can have unexpected consequences on offspring development and physiology and this effect can be seen across multiple generations. For example, having parents without a microbiome leads to gene expression changes in offspring that can persist for multiple generations. While these studies demonstrate the microbiome has an impact on a host physiology and development, they attribute varying bacterial strains and host signaling pathways to these phenotypes. Thus, we still know little about specific mechanisms by which the microbiome impacts early development or how it contributes to the faithful maintenance of processes like gonadogenesis and early embryogenesis.

To address some of these questions, this work combines fecundity assays and immunostaining to explore the impact of the microbiome on male and female gonadogenesis and early embryogenesis. When flies are reared without a

microbiome (axenic), females lay fewer eggs over time compared to their conventionally reared counterparts. Axenic female flies also tend to have smaller and more immature ovaries, where many ovarioles do not contain late-stage oocytes. Taken together, these data suggest that the microbiome plays a role in fecundity and oogenesis. Oogenic mutations will be looked at following these results for phenotypes that phenocopy what is seen in axenic females. Overall, this research links the microbiome to fundamental developmental processes which could mean that this same relationship exists in other organisms.

434V **Functional assembly of the large and unique germ granule during germline development in the wasp *Nasonia vitripennis*** Kabita Kharel[1], Samuel J Tindell[1], Allie Kemph[2], Ryan Schmidtke[1], Emma Alexander[1], Jeremy Lynch[2], Alexey L Arkov[11]Biological Sciences, Murray State University, [2]Biological Sciences, University of Illinois at Chicago

During germ cell development, Drosophila assembles multiple small RNA-protein granules at the posterior pole of the oocyte referred to as polar granules. However, a distantly related wasp Nasonia forms a very different single germ granule, called oosome, which is about 40 times larger than a Drosophila polar granule. After its assembly, the oosome migrates in the early embryo's cytoplasm, eventually arriving at the posterior pole, where it is fragmented during its segregation into primordial germ cells which form at the pole. Using molecular, proteomics and super-resolution microscopy imaging approaches, we are providing insights into the molecular mechanisms of the assembly and function of this unique germ granule. Interestingly, unlike the fly, the wasp utilizes alternatively spliced RNA-helicase Vasa isoforms during germline development and oosome formation. In addition, while other conserved components of germ granules, such as Oskar, Aubergine and Tudor proteins are recruited to the oosome, these polypeptides show a distinct and specific localization within the oosome, which is different from Drosophila polar granules. Our results point to the high degree of plasticity in the assembly of membraneless organelles and suggest that novel molecular features of conserved proteins and the oosome-specific components result in the assembly of this large structure.

435V **A transcriptomics based RNAi screen for components of the synaptonemal complex in *Drosophila*** Cynthia Staber[1], Salam Briggs[1], Andrew Price[1], Scott Hawley[1,21]Stowers Institute for Medical Research, [2]2Department of Molecular and Integrative Physiology, University of Kansas Medical Center

The synaptonemal complex (SC) is the structural lattice that connects homologous chromosomes during meiosis I in *Drosophila* females. Mutants that lack SC do not form double strand breaks (DSBs) and have increased rates of chromosome non-disjunction. While the SC is structurally conserved across species, the proteins that make up the SC are rapidly evolving, making homology-based searches quite difficult. Only four structural components of the SC have been identified in Drosophila. More have been identified in yeast and *C. elegans* making us think we are missing components. Two labs recently published single-cell RNA-seq (scRNA-seq) data sets from *Drosophila* ovaries. We have reanalyzed the data from both groups to identify germ cells expressing known SC component transcripts. We reasoned that those cells would also express yet unidentified SC component transcripts. Our analysis generated a list of candidate genes that could be rapidly screened by RNAi using lines available from the Bloomington Drosophila Stock Center. RNAi lines were individually crossed to a nanos-Gal4 driver to express the RNAi hairpin and knock down the expression of each gene in the female germline. Ovaries from the knockdown females were dissected and stained with antibodies to C(3)G to identify the SC and Orb to determine ovary specification and identify developmental defects. While our overall goal was to identify genes affecting the SC, we also included the antibody γH2Av to mark sites of DSBs as this would allow us to simultaneously screen for genes that affected DSB formation or resolution. Our analysis identified all four known SC structural genes. Defects in c(3)G, corolla, and c(2)M were verified by RNAi. New candidate genes include *Lamtor5* and *stet* which affect SC formation and *Ref1* and *MAGE* which have SC maintenance defects. We are analyzing these and other candidate genes to determine their role in SC structure and/or function.

436V **A multi-protein complex regulates *cycB* translation in the *Drosophila* male germline** Catherine C Baker, Lorenzo Gallicchio, Margaret FullerDevelopmental Biology, Stanford University School of Medicine

The *Drosophila* male germline contains both mitotic cells (spermatogonia) and meiotic cells (spermatocytes), and the regulation of cell division in these two cell types is dramatically different. Spermatogonia divide regularly and efficiently; spermatocytes, in contrast, undergo a meiotic G2 prophase that lasts 3.5 days, and the concurrent delay of the meiotic divisions is mediated by fine-tuned control of the temporal expression of core cell cycle components. One such cell cycle factor is Cyclin B (CycB). CycB protein expression is high in mitotic spermatogonia, and then very low in immature spermatocytes. CycB protein levels spike again just before spermatocytes enter the meiotic divisions. Published work from our lab has shown that the RNA-binding protein Rbp4 and its co-factor Fest repress *cycB* translation, mediated by

sequences in the 130nt *cycB* spermatocyte 3'UTR. Subsequent work has revealed that a third protein (Lutin/CG1690) associates with Fest and Rbp4 through an RNA-independent interaction with Fest. Lut is required for repressing *cycB* translation, although the premature expression of CycB protein in a *lut* mutant appears to begin later than it does in an *rbp4* mutant. We confirmed this difference in timing by using the heat-shock time-course developed in the lab, where *bam* mutant spermatogonia are given a pulse of wild-type Bam protein under the control of a heat-shock promoter, then differentiate into spermatocytes and later stages in synchrony. CycB protein is high by 102h post-heat-shock (PHS) in wild type, by 94h PHS in *lut*, and by 54 hours PHS in *rbp4*. In addition, we found that *rbp4* and *lut* spermatocytes enter the meiotic divisions about 6 and 8 hours earlier than wild type, respectively. These data indicate that precocious CycB expression is permissive but not sufficient to drive early meiotic entry. Furthermore, we have found that Syp is required for CycB accumulation in mature spermatocytes. Syp, like Rbp4, binds the 130nt *cycB* 3'UTR. Syp also binds to Fest in the absence of RNA and can co-precipitate with both Rbp4 and Lut in the presence but not absence of Fest. Binding of Syp, Rbp4, and Lut to Fest does not change in early (72h) vs. late (104h) spermatocytes, suggesting that the composition of the complex is static. Nevertheless, genetic epistasis tests using the *rbp4 syp* and *lut syp* double mutants indicate that Syp may repress Rbp4 activity, and that Lut may in turn inhibit Syp or may repress *cycB* translation in parallel to Rbp4.

437V **Investigating the development of key somatic cells in the Drosophila ovary** Joanna Portillo[1], Abigail Dove[2], Mark Van Doren[21]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 Transcription factors (DMRTs). 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, since this process occurs early in development and there are relatively few cell types present, 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 cell and support cells of the ovary form and attain their distinct identities. Our current theory is that the somatic support cell, Escort Cells (EC), and the somatic stem cell, Follicle Stem Cells (FSC), develop from a set of somatic cells known as "intermingled cells" (ICs) in the 3rd instar larval (L3) ovary. Previous work by the Lehmann lab demonstrated that the ICs in the larval gonad have distinct anterior vs. posterior identities, and that the posterior cells are partially biased toward FSC fate. 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. Further, 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 how larval ICs become specified into anterior vs. posterior identities and how this impacts formation of ECs and FSCs. We are investigating two hypotheses: 1) that differences in IC identity arise from differences in Hox gene expression in the somatic gonad and 2) that extrinsic signaling pathways control differences in IC identity and formation of ECs vs. FSCs. In particular, precursors of the somatic gonad express either abdA alone, or abdA and AbdB, and we will test if manipulating this Hox code alters IC identity. In addition, we are studying the role of the Hh and Wnt pathways in patterning ICs at larval stages, and how this is affected by the Jak/Stat pathway in pupal stages when FSC precursors arise. These studies will inform us about how important cell types in the ovary develop and allow us to understand how Dsx creates the distinct developmental programs of the ovary and testis.

438V **Inducible *Gal4* expression reduces egg production and disrupts normal morphology in the *Drosophila* ovary** Tiansheng Zhang, Niyoosha Abdollahpour, Kristopher McConnellNatural Sciences, University of Wisconsin-Superior

The Gal4/UAS system is a powerful tool that is widely used for transgene expression in *Drosophila* genetic analysis. Despite the widespread use of Gal4/UAS and its many variations, the effect that Gal4 itself has on the biology of the fruit fly is poorly understood. Furthermore, the effects that Gal4 may have specifically on fecundity have not been addressed. We used the heat inducible *Hsp70:Gal4* construct to investigate the effect of Gal4 on egg production and oogenesis in the *Drosophila* ovary. We observed that *Hsp70:Gal4* expression causes female fruit flies to stop laying eggs for several days after a single heat shock. In heterozygous *Hsp70:Gal4/+* flies, egg production ceases, but is restored by the third day after heat shock. This disruption of egg production is dependent on the transcriptional activity of Gal4, as $Hsp70{:}Gal4^{DBD}$ flies continue to lay eggs after heat shock. We observed similar disruptions in the progesterone inducible GeneSwitch Gal4 ($Act5C{:}Gal4^{SwitchPR}$). Microscopic analysis of *Hsp70:Gal4* ovaries showed cell death in both follicle cells and nurse cells after heat shock, as evidenced by the presence of pyknotic nuclei and caspase staining. This evidence of cell death persisted up to four days post heat shock. In addition, *Hsp70:Gal4* ovaries also displayed altered morphology and what appears to be an accumulation of epithelial sheath tissue. Paradoxically, fly strains with ubiquitous expression of Gal4 (such as *Act5C:Gal4)* can be maintained as stable lines, suggesting that any effects on fecundity in these lines are modest. We observed that coexpression of *Act5C:Gal4* enhances the egg production defects in *Hsp70:Gal4/+* heterozygotes. We

observed similar enhancement in *Hsp70:Gal4/+* heterozygotes with coexpression of developmentally patterned Gal4 lines, including *c323:Gal4*, which expresses Gal4 in follicle cells from Stages 9-14. Our research illustrates the need for caution when interpreting results to ensure that any effects observed after transgene expression are not due to Gal4 expression alone.

439V **Insights into the mechanistic basis of the manipulation by the endosymbiotic bacteria *Wolbachia pipientis* of female reproduction in *D. melanogaster*** Catherine Kagemann[1], Charles Aquadro[21]Molecular Biology and Genetics, Cornell University, [2]Molecular Biology and Development, Cornell University

Wolbachia pipientis are maternally transmitted endosymbiotic bacteria commonly found in arthropods and nematodes. *W. pipientis* have complex interactions with their hosts, and many of these interactions serve to increase transmission. *W. pipientis* commonly manipulate reproduction of the host via cytoplasmic incompatibility, resulting in embryonic mortality. Additionally, *W. pipientis* rescues the *bag of marbles* (*bam*) partial loss of function (hypomorph) fertility phenotype in female *Drosophila melanogaster*. *Bam* is an important germline stem cell (GSC) gene involved in GSC renewal and cystoblast differentiation. GSCs are required to produce egg and sperm, making the genetic interaction between *W. pipientis* and GSC genes such as *bam* of great evolutionary interest to us. While we understand that *W. pipientis* contributes to the rescue of the *bam* hypomorph phenotype, we aim to determine the functional mechanisms that are behind this interaction. Our results show that rescue of the mutant *bam* phenotype depends on the genotype and titer of *W. pipientis* and the magnitude of rescue is dependent on the age of the female fly. Our RNA-seq analysis revealed that W. pipientis infected *Drosophila* differentially express many of *bam's* genetic and physical interactors in the *bam* hypomorph genotype, but not *bam* itself. Differential expression of *bam's* interactors does not significantly differ between *W. pipientis* variants infecting *D. melanogaster* and thus fails to explain the difference in *bam* hypomorph fertility rescue we have observed among *W. pipientis* variants. Interestingly, *w*MelCS-like *Wolbachia* variants (with higher titer and *bam* hypomorph fertility rescue) cause expression of over 100 GSC genes in flies before mating while the *w*Mel-like variants (with lower titer and *bam* hypomorph fertility rescue) enrich for expression of genes involved in oogenesis and reproduction in mated three- and six- day old flies.

440V **The *Wolbachia* interaction with the *D. melanogaster bag of marbles* (*bam*) gene is specific to *bam* function** Miwa Wenzel[1], Charles F Aquadro[21]Cornell University, [2]Molecular Biology and Genetics, Cornell University

The D. melanogaster protein coding gene bag of marbles (bam) plays a key role in early male and female reproduction by forming complexes with partner proteins to promote differentiation in gametogenesis. Like another germline gene, Sxl, bam genetically interacts with the endosymbiont Wolbachia, as Wolbachia rescues the reduced fertility of a bam hypomorphic mutant. Here, we explored the specificity of the bam Wolbachia interaction by generating 22 new bam mutants. We find Wolbachia rescues all six mutants of partially reduced fertility, but none of the four with severely reduced fertility. There is no specificity between the rescue and the known binding regions of bam, suggesting Wolbachia doesn't interact with one singular bam partner to rescue fertility. Fertility assessment of a bam RNAi knockdown mutant reveals that Wolbachia rescue is specific to functionally mutant bam alleles as the partially fertile bam knockdown is not rescued by Wolbachia. Consistent with this, we find no substantial evidence of Wolbachia interaction with germline stem cells in bam mutants. As bam and Sxl have been proposed to function together, it is possible that Wolbachia is rescuing the bam and Sxl hypomorphs through the same molecular mechanisms that target restoration of their protein complexes' functions. Intriguingly, we also find that Wolbachia rescues the fertility of a male bam hypomorph and are interested in further studying this to see if there are commonalities in the Wolbachia interactions of males and females.

441V **Is Roundup-Induced Reproductive Toxicity Reversible in Drosophila Melanogaster?** Kalinah A Winston, Becky TalynDepartment of Biology, California State University San Bernardino

Herbicides are used worldwide to protect crops and kill unwanted vegetation. The use of these herbicides, however, has exposed non-target organisms to harmful chemicals. Studying how exposure to these herbicides can shed light on how certain chemicals harm humans. The present study investigates how exposing Drosophila melanogaster to Roundup® will affect reproduction. This study was conducted by first exposing flies to either control medium or medium treated with Roundup® for one week then moved to a new vial containing one of these in a 2X2 design. Mortality and reproduction was noted for each trial. Preliminary data shows that flies treated with Roundup for both weeks have less larvae and pupae and high mortality. Data also shows that flies treated with Roundup® and then treated with control medium regain reproductive capabilities. Conducting this research will contribute to a better understanding of how herbicide exposure harms the human reproductive system.

442V **Ectopic expression of ovary expressed *polo* transcripts and duplicates in *Drosophila melanogaster* testis creates female-biased sex ratios** Paola Najera, Olivia A Dratler, Alexander B Mai, Miguel Elizarraras, Rahul Vanchinathan, Christopher A Gonzales, Richard P MeiselBiology and Biochemistry, University of Houston

Polo-like kinases (Plks) play vital roles in mitosis and meiosis. *Drosophila melanogaster* has a single Plk gene (*polo*) that is alternatively spliced into two transcripts that differ in their 5'- and 3'-UTR, but not in their coding sequence. Curiously, *polo* has also been duplicated multiple times within the evolution of the *Drosophila* genus. In *D. pseudoobscura*, the ancestral *polo* gene (*Dpse-polo*) is found on a neo-X chromosome, and there are two autosomal duplications (*Dpse-polo-dup1* and *Dpse-polo-dup2*) that are predominately testis expressed. The precise roles of the *polo* transcripts and duplications in male meiosis are unknown. Here, we show that when the *D. melanogaster* ovary-derived *polo* mRNA or *Dpse-polo* mRNA is expressed in *D. melanogaster* testis, the number of female offspring is greater than the number of male offspring. In contrast, equal numbers of males and females are produced when testis-derived *polo* mRNA or *Dpse-polo-dup1* are expressed in the *D. melanogaster* male germline. These results suggest that *polo* duplicates and transcripts are specialized for sex-specific meiotic functions, specifically related to equal inheritance of the X and Y sex chromosomes in the male germline. Consistent with such specialization, we find that *Dpse-polo-dup1* has accumulated significantly more amino acid substitutions than *Dpse-polo* since the duplication event. We hypothesize that the sex-specific specialization of *polo* duplicates and transcripts may be related to repeated intragenomic conflicts involving segregation distorters that differentially affect spermatogenesis and oogenesis.

443T **Revealing hidden micropeptide functions using high-throughput screening** Emma L Walmsley[1], Shahzabe Mukhtar[1], Gabriel N Aughey[2], Tony D Southall[11]Life Sciences, Imperial College London, [2]University College London

A hidden world of biological regulation exists in the form of micropeptides, which are defined as polypeptides of 100 amino acids or less that are produced from small opening reading frames (smORFs). Despite their small size, micropeptides have been shown to have important cellular roles in many organisms including *Drosophila melanogaster*. Analysis of genomes and proteomes has indicated the existence of thousands of uncharacterised smORFs, but they remain largely understudied due to a lack of methods for identifying micropeptide function. Here, we aim to address this gap in the field by using a cutting edge high-throughput yeast two-hybrid method to simultaneously screen for the interacting partners of 384 smORF-encoded micropeptides which previous studies have shown are present in *Drosophila melanogaster* embryos. We screened over 30 million binary micropeptide-protein pairs, resulting in the identification of thousands of putative micropeptide-protein interactions. These interactions are highlighting micropeptides which bind to proteins associated with a diverse range of biological processes including metabolism, gene expression regulation, and development. We anticipate this screening method will advance not only our understanding of the biological processes being regulated by each micropeptide, but also offer a crucial way to advance the emerging field of smORF research.

444T **Defining Activities of the C-terminus of KDM5 Essential to Development and Viability** Julie Secombe, Melissa CastiglioneAlbert 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. Most work to-date has focused on the histone demethylase activity of KDM5 proteins, which targets the active chromatin mark H3K4me3. However, KDM5 proteins can also regulate transcription through non-enzymatic mechanisms. While KDM5 is essential for development, its demethylase activity is not required, as is demonstrated by the viability of demethylase-dead adult flies. In this work, we will examine essential functions of KDM5 via a novel truncation allele, *kdm5*^{Q19}, which does not alter demethylase activity. *kdm5*^{Q19} inserts a stop codon in a previously unrecognized, evolutionarily conserved, motif within an intrinsically disordered region of KDM5 at the C-terminus. *kdm5*^{Q19} animals do not survive to adulthood, which is distinct from null, demethylase dead, and other mutants generated in our lab, suggesting that the motif disrupted by the truncation has an essential as-yet-unknown role in normal KDM5 function. 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.

445T **Targeting Dosage Compensation to the Drosophila Male X-chromosome** Angelica Aragon Vasquez, Claire Gray, Melissa Aldana, Joseph Aguilera, Mukulika Ray, Erica LarschanBrown University

Sex differences in response to clinical therapeutics are common but little is understood about the underlying mechanisms. Therefore, a deep understanding of male versus female gene regulation is essential to reveal these mechanisms. As a model for understanding sex differences in gene regulation, we are investigating how the Drosophila single male X-chromosome achieves its essential sex-specific role of upregulating all its active genes approximately two-fold, a phenomenon known as dosage compensation. A key pioneer transcription factor, Chromatin Linked Adaptor for MSL Proteins (CLAMP), is critical in targeting dosage compensation to the male X-chromosome. However, CLAMP is also found on all chromosomes in the male and female genomes. I hypothesize that CLAMP works alongside other cofactors to form a specific chromatin environment which helps target the Dosage Compensation Complex (DCC) specifically to the male X-chromosome. By performing Cleavage Under Targets and Release Using Nuclease (CUT and RUN), I will map all CLAMP and DCC binding sites in both male and female larvae for wild-type and clamp mutant lines. Preliminary data has shown there is a loss in wild-type DCC binding but an increase in ectopic autosomal DCC binding in clamp mutants. After identifying novel loci via CUT and RUN, I will then perform bioinformatic analyses, MEME and R-cis-target, which will reveal novel motifs and cofactors involved in X-chromosome targeting of dosage compensation. My research will provide novel insight into sex differences in gene regulation, which will shed light on how future therapeutics will affect males and females differently.

446T **Mechanism of the anterior determinant gene *panish* in the midge *Chironomus riparius*** Muzi Li, Urs Schmidt-OttThe University of Chicago

We use primary axis specification in embryos of flies (Diptera) as a model to examine the molecular basis of evolutionary coherence and plasticity in developmental gene networks. Dipteran insects use localized maternal mRNAs, also known as anterior determinants (ADs), to determine the embryo's anterior end. At the beginning of embryogenesis, these mRNAs generate transcription factor gradients that regulate downstream segmentation genes in a concentration-dependent (morphogen-like) manner. Surprisingly, distinct anterior determinants have been found in fly species representing different lineages. *Drosophila melanogaster* uses a homeodomain protein (Bicoid) to drive anterior-specific gene expression via chromatin accessibility changes and transcriptional activation, but whether the unrelated ADs of other dipteran species function through comparable mechanisms and sets of target genes remains unknown. To address these questions, we are studying the AD of the midge *Chironomus riparius*. Chironomus lacks *bicoid* and uses a newly evolved chironomid-specific cysteine-clamp gene (*panish*) to establish anterior patterning during early development. *panish* is a highly diverged paralog of *Tcf/pangolin* (also known as *pan*; hence the name *pan*"ish"), a prominent component of the Wnt signaling pathway but has no similarity with *Tcf/pangolin* outside the cysteine-clamp domain. We are testing whether Panish plays a role in shaping chromatin accessibility during early zygotic genome activation as a means of breaking axial symmetry and seek to identify direct targets of Panish. We expect this project will help to understand co-option mechanisms underlying the evolutionary plasticity of developmental gene networks in embryos.

447T **Investigating intersecting RNA regulatory roles for Glorund in the ovary and the adult brain** Brooke Hull, Elizabeth R GavisMolecular Biology, Princeton University

Post-transcriptional regulation by RNA-binding proteins (RBPs) plays an important role in generating spatial and temporal patterns of gene expression during animal development. RBPs regulate numerous transcripts, but for many RBPs, these targets and their post-transcriptional regulation remains unknown. Glorund (Glo), the *Drosophila* homolog of the hnRNP F/H family of RNA binding proteins, was identified through its role during oogenesis to control translation of *nanos* RNA and is also implicated in regulation of splicing. In addition to these oogenesis functions, Glo is highly expressed in the adult brain where neither its targets nor its regulatory functions are known. Glo has three RNA binding domains, each of which can recognize at least two different RNA sequence/structural motifs, using two distinct RNA binding surfaces. Genetic analysis showed that some functions of Glo require only one binding surface, whereas other functions require both, suggesting that subsets of Glo target RNAs may have different binding interactions. Using a genome wide approach called HyperTRIBE, we are identifying Glo target RNAs in both the adult brain and the ovary, where Glo is most highly expressed. Expression of Glo is fused to the catalytic domain of the RNA editing enzyme ADAR results in editing of transcripts bound by Glo, which can then be detected by RNA-seq. Preliminary work identified targets enriched in synaptic processes, neuron projection extension, and oogenesis. Ongoing investigation focuses on the intersection between putative Glo targets in the adult brain and the ovary. Validation and investigation of shared targets will help us understand how functionally distinct tissues can be established through the regulation of the same RNAs. Additional

work seeks to identify protein interactors using proximity labeling to build a mechanistic view of RNA regulation by Glo. Defining the roles of Glo in oogenesis and in the nervous system will provide insight into the functional diversity of RNA-binding proteins and their importance during development, as well as inform how dysregulation of RNA metabolism contributes to disease.

448T **The histone acetyltransferase Nejire is recruited to the genome by the pioneer factor Zelda, and is required to activate gene expression during the maternal-to-zygotic transition** Audrey J Marsh[1], George Hunt[2], Sergei Pirogov[2], Abby Ruffridge[1], Tyler Gibson[1], Katherine Hullin[1], Mattias Mannervik[2], Melissa Harrison[11]University of Wisconsin-Madison, [2]The Wenner-Gren Institute

The sperm and egg are differentiated cell types, individually specialized for the purpose of fertilization. After merging to form the diploid genome, the embryo must remove signatures in the parental genomes to enable reprogramming to a totipotent state. Reprogramming is essential for the embryo to ultimately differentiate into all cell types of the adult organism. This reprogramming process occurs during a period called the maternal-to-zygotic transition (MZT). Initially transcriptionally silent, the zygotic genome is activated by maternally encoded factors loaded into the oocyte prior to fertilization. Activation of the genome is controlled by a specialized set of transcription factors called pioneer factors, defined by the ability to bind and increase chromatin accessibility. In Drosophila melanogaster, the pioneer factor Zelda (ZLD) is an essential activator of zygotic transcription. Prior to ZLD-mediated genome activation, chromatin is largely comprised of nucleosomes devoid of post-translational modifications. Intriguingly, ZLD-bound sites are enriched with active histone acetylation marks, suggesting that a histone acetyltransferase (HAT) is functioning with ZLD to induce transcription. We showed that during the MZT Nejire (NEJ), the Drosophila homologue of the HAT family p300/CBP, is bound to thousands of loci also occupied by ZLD. Maternal depletion of ZLD revealed recruitment of NEJ and histone acetylation is dependent upon ZLD at shared sites. Expression of ZLD in cell culture is sufficient to induce accessibility and increase histone acetylation at bound loci. We are investigating whether NEJ mediated histone acetylation is essential for accessibility at ZLD sites. Our data suggests that ZLD drives activation of the genome, in part, by recruiting NEJ to acetylate histones and maintain accessibility at ZLD occupied sites. We optimized the CRY2-optogenetic system to inactivate NEJ during widespread genome activation and coupled this with RNA-sequencing. We found maternally encoded NEJ is essential for progression through the MZT and for the normal expression of hundreds of genes. Ongoing work will identify whether ZLD is directly recruiting NEJ or additional factors are necessary to recruit NEJ to ZLD-bound loci. Together with data from vertebrates our work supports a model in which a conserved mechanism for genome activation involves pioneer factors that prime the genome for activation by recruiting HATs, such as NEJ, to cis-regulatory sites.

449T **The role of Piwi in selecting transcription start sites** Jiaying Chen[1], Na Liu[2], Haifan Lin[21]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. 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 in the *Drosophila* ovary upon loss of Piwi and termed these genes as Altered TSS Usage (ATU) genes. Combining single-cell RNA sequencing data of wildtype fly ovaries and quantitative PCR validations in germline-specific and soma-specific *piwi*-knockdown fly ovaries, we found Piwi can regulate TSS usage in the germline or somatic cells, or both. Bioinformatic analysis revealed no differential targeting of Piwi-interacting RNAs (piRNAs) on ATU genes versus non-ATU genes, indicating this Piwi-dependent TSS usage regulation is not guided by piRNAs. RNA Polymerase II (Pol II) ChIP-seq data of germline-specific *piwi*-knockdown ovaries revealed that Piwi affects Pol II density and binding profile at TSSs of ATU genes. Mass spectrometry of Piwi-immunoprecipitated interactors in the nuclear fraction of fly ovaries revealed several novel interactors. Based on these findings, we propose that Piwi regulates TSS usage in *Drosophila* ovaries via interaction with epigenetic regulators in a piRNA-independent fashion.

450T **Quantitative analysis of the roles of IRM cell adhesion molecules in column formation in the fly brain** Yunfei Li[1], Miaoxing Wang[2], Kousuke Imamura[3], Makoto Sato[31]Developmental Neurobiology, Kanazawa University, [2]Kanazawa univercity, [3]Kanazawa University

The visual center of fly shares columnar and layered structures with the mammalian cerebral cortex. The columns are the basic structural and functional units of the brain made from multiple neurons. However, developmental mechanisms of

the columns are largely unknown.

Irre Cell Recognition Module (IRM) is a family of immunoglobulin cell adhesion molecules. Antibody staining of the four fly IRM proteins revealed that Roughest (Rst), Kirre (Kin of Irre), Hibris (Hbs) and Stick-and-stones (Sns) are localized to the columns of the pupal medulla, the largest ganglion of the fly visual center. The medulla column shows a donut-like structure as visualized by the localization of Flamingo (Fmi) and the axon terminals of the core columnar neuron, Mi1. The donut-like columnar structure was destroyed in the mutants of rst, kirre, hbs and sns, suggesting that the four IRM proteins are essential for column formation.

Since IRM proteins are heterophilic cells adhesion molecules, they may regulate cell adhesion between columnar neurons. To test this possibility, we specifically knocked down rst, kirre, hbs and sns in Mi1 neurons, and examined the defects in Fmi distribution.

The morphology of the columns was quantified using a custom-made image processing program. From original confocal 3D images of control and RNAi backgrounds, individual column images were automatically extracted and quantified. The results showed that the smoothness of the columns were decreased when one of IRM genes was knocked down in Mi1, suggesting that four IRM genes play essential roles in Mi1 neurons to regulate the column shape. We also show that their expression in the other columnar neurons, Mi4 and T4/5, is essential, suggesting that the interactions between IRM proteins and multiple neurons shape the columns in the fly brain.

451T **Modulation of DNA-protein binding reveals mechanisms of spatiotemporal control in early embryonic development** Sahla Syed[1], Bomyi Lim[21]University of Pennsylvania, [2]Chemical and Biomolecular Engineering, University of Pennsylvania

It is well known that an enhancer regulates the spatiotemporal expression of its target gene through recruiting activators and repressors (transcription factors) to the cognate binding motifs in the region. While an enhancer may contain multiple weak binding sites for the same transcription factors (TFs), the role of these sites and their specific spatial arrangement in affecting the overall transcriptional competency of the enhancer has yet to be fully understood. In this study, we have implemented the MS2-MCP live imaging technique to quantitatively analyze the regulatory logic of the snail distal enhancer in early embryos. Through systematic modulation of 3 Dorsal (Dl) and 1 Twist (Twi) binding motifs, we found that the mutations in any one of these binding sites cause a drastic reduction in the average transcriptional intensity, resulting in a reduction in total mRNA production. We provide evidence of synergistic capabilities of each binding site, such that multiple binding sites with moderate affinities cooperatively recruit more TFs to drive stronger transcriptional activity. In addition, our HMM-based stochastic modeling of transcriptional activity suggests that embryos with binding site mutations have a higher probability of remaining in the inactive promoter state, supporting our observation of reduced transcriptional amplitude and mRNA production. Through a combination of experimental and theoretical approaches, we propose distinct mechanisms by which TFs regulate spatial and temporal gene expression dynamics and drive robust pattern formation during early embryonic development.

452T **Visualization of a temporal sequence of upstream events leading to promoter activation in the early embryo** Chun-Yi Cho, Patrick H. O'FarrellBiochemistry and Biophysics, University of California, San Francisco

The pioneer transcription factor (TF) Zelda plays a major role in activating the early wave of zygotic gene expression in *Drosophila* embryos, yet the exact mechanisms remain unclear. We present evidence that Zelda initiates a regulatory cascade during promoter activation at the beginning of interphase. Zelda acts in conjunction with the lysine acetyltransferase dCBP to recruit the bromodomain-containing coactivator dBrd4, which then triggers abrupt clustering of RNA polymerase II (RNAPII). Live imaging reveals the sequential and transient recruitment of these proteins to discrete clusters after mitosis, suggesting a stepwise modification of nuclear microenvironments associated with activating genes. Knockdown of Zelda, dCBP, or dBrd4 establishes that a hierarchy of dependency underlies their temporally ordered recruitment. A negative feedback loop disperses clusters of dBrd4 and RNAPII when significant nascent transcript accumulation occurs. Our results suggest that activation of transcription by eukaryotic TFs involves a succession of distinct biochemical complexes, with the successful, but rare, transition through multiple states ultimately fueling a self-limiting burst of transcription at selected loci.

453T **Dynamics of recruitment of transcription elongation factor SPT6 to the histone locus body during the activation of replication dependent histone genes in early Drosophila embryogenesis** Mia C Hoover[1], James P

Kemp Jr.[2], Robert J Duronio[31]Biology, University of North Carolina, [2]Integrative Program for Biological and Genome Sciences, University of North Carolina, [3]Biology, Integrative Program for Biological and Genome Sciences, Lineberger Comprehensive Cancer Center, Department of Genetics, University of North Carolina

Histones are essential for packaging DNA, and their expression is tightly controlled and coupled to DNA replication. In Drosophila, replication-dependent (RD) histone mRNAs are transcribed from a single locus at which an evolutionarily conserved, phase-separated nuclear body forms — the histone locus body (HLB). Here, using antibodies and fluorescently tagged proteins we investigate the spatiotemporal localization of the transcription elongation factor SPT6 and RNA Pol II at the HLB in developing Drosophila embryos. By combining this approach with a sensitive FISH probe that can detect nascent RD histone transcripts, we examined the relationship between the localization of these factors and actively transcribing RD histone genes. From imaging both live and fixed embryos, we found that SPT6 is continuously enriched at the HLB throughout S phase of the syncytial nuclear cycles 11-13, yet is only transiently present at the HLB during cycle 14. SPT6 is enriched at the HLB for ~10 min at the beginning of nuclear cycle 14, with no noticeable enrichment as the cells finish S phase and enter G2 for the first time. In contrast, RNA Pol II and nascent histone RNA remain present at the HLB throughout all of cycle 14, showing an unexpected uncoupling of the association between SPT6 and RNA Pol II during active transcription. Probes recognizing only the 5' or the 3' ends of the RD histone genes indicate that RNA Pol II is not paused but still actively transcribing throughout cycle 14. These data suggest that for most of S phase and G2 SPT6 is not needed for production of histone mRNA. Our next steps include using the JabbaTrap system to remove SPT6 from the HLB in the developing embryo to determine whether it is necessary for transcription of RD histone genes or localization of HLB factors.

454T **Multiple domains in transcription factor CLAMP regulates sex-specific splicing during Drosophila development.** Pranav Mahableshwarkar, Mukulika Ray, Erica LarschanMolecular Biology, Cellular Biology, and Biochemistry, Brown University

Chromatin-linked adaptor for male-specific lethal (MSL) proteins (CLAMP) is a maternally deposited transcription factor and DNA/RNA binding protein that regulates dosage compensation in males and early embryonic sex-specific splicing in both males and females. However, the question of whether CLAMP also regulates sex-specific splicing during later development remained. Although earlier studies in the lab showed CLAMP binds to spliceosome components, the role CLAMP plays in the function of the splicing complex remains unclear. Since understanding the role of a TF like CLAMP in post-transcriptional RNA processing would help reveal mechanisms of co-transcriptional splicing - an important component of gene regulation - we investigated the role of CLAMP in sex-specific splicing and DNA/RNA binding throughout development.

Analysis of RNA-seq data from cell lines and whole third instar larvae (L3) using the Larschan Lab's time2splice pipeline revealed that 60-80% of CLAMP-dependent alternative splicing is regulated in a sex-specific manner. To understand the context-specific role of CLAMP, RNA sequencing data from mutants with deletions in the glycine-rich PrLD and MSL2 binding domains of CLAMP were analyzed. The MSL2 protein is involved in dosage compensation in males and mostly affects unique sex-specific splicing events.

All CLAMP mutants gave rise to new sex-specific splicing events when compared to control samples - supporting CLAMP's role of inhibiting aberrant splicing. Specifically, the PrLD domain was found to be important in CLAMPs splicing functionality. Interestingly, CLAMP regulates female sex-specific splicing of spliceosome component Hrp38, resulting in different isoform expression. The human homolog hnRNPA2B1 is known to be involved in neurodegenerative diseases. Using qRT-PCR and RT-PCR, splicing events in a set of candidate genes regulated by CLAMP, including Hrp38, were validated.

To understand the mechanism of co-transcriptional splicing regulated by CLAMP's binding to DNA and RNA molecules, we developed scripts to computationally analyze integrated CLAMP DNA binding (CutnRun) and RNA binding data (iCLIP) sets to determine whether CLAMP's RNA binding property is interdependent or independent of its DNA binding properties. We discovered a correlation between RNA/DNA bindings sites relating to differential splicing events.

My analysis additionally identified several new sex-specific splicing events in genes involved in important biological processes that could be interesting to study in the future.

455T **Characterizing the transcriptional landscape of *Drosophila melanogaster* centromeres** Asna Amjad[1], Luoxuan Ouyang[1], Savannah Hoyt[1], Cecile Courret[2], Ryan Drennan[3], Luke Wojenski[3], Charles Limouse[4], Amanda Larracuente[2], Rachel O'Neill[1], Barbara Mellone[11]Molecular and Cell Biology, University of Connecticut, [2]Department of Biology, University of Rochester, [3]University of Connecticut, [4]Department of Biochemistry, Stanford University School of Medicine

Transposable elements and satellite DNA are major components of complex centromeres and transcripts originating from centromeric sequences have been observed across a broad range of species. However, the transcriptional landscape of complex centromeres remains largely unexplored including due to lack of annotated centromeres in most genome assemblies. In *Drosophila melanogaster*, centromeres consist of islands of complex DNA enriched in retroelements flanked by satellite repeats. Each centromere has a distinct size and is composed of a unique arrangement of several DNA elements that are present also elsewhere across the genome. A non-long terminal repeat (non -LTR) retroelement *Jockey-3* is the only repeat shared amongst all *D. melanogaster* centromeres and is a conserved centromere element in *D. simulans*, suggesting a conserved role for this element. To better understand the full extent of repetitive elements transcription in *D. melanogaster*, we performed Precision Run-On sequencing (PRO-seq) to capture nascent transcripts and total RNA-seq in *D. melanogaster* larval brains and embryos. Nascent transcripts analysis in embryos showed that the *Jockey-3* element is active, but traditional mapping methods to determine centromere derived transcripts proved to be unfeasible. To overcome the challenges of short read length and multi-locus mapping in the highly repetitive centromeric regions, we used a k-mer analysis approach and several mapping methods for both PRO-seq and RNA-seq data to identify transcripts emanating from the centromeres. We identify noticeable differences between nascent and stable transcripts. In PRO-seq data, we detect nascent transcription of *Jockey-3* and other retroelements at the centromeres with very little nascent transcription from satellite repeats. In contrast, more satellite repeats transcripts are observed in total RNA samples. This suggests that satellite repeats reflect stable transcripts while retroelement transcripts may be less stable yet continuously transcribed. Statistical analyses show more read overlapping for full-length *Jockey-3* copies compared to truncated copies, while not much difference is observed between centromeric vs. non-centromeric copies of *Jockey-3*. We are also using a mapping-independent approach to identify reads originating from specific repeat elements to detect low levels of satellite transcription. These analyses will help to further explore the function of centromere transcription in *D. melanogaster*.

456T **Defining the Dynamics of Transcriptional Bursting in Developing *Drosophila* legs** Rina HeltBiology, Johns Hopkins University

Transcription is a dynamic process during development. Much of our understanding of transcription comes from studies of snapshots of individual timepoints. Live imaging approaches based on MS2-MCP technologies have provided numerous insights into transcriptional dynamics during embryonic development, yet we still know very little about how these dynamics affect later developmental processes. Studying transcriptional bursting in genes that regulate development, such as *spineless* (*ss*) in *D. melanogaster*, can reveal the role of transcriptional dynamics in the production and regulation of specialized appendages and organ systems. *ss* is differentially expressed in the eye, antenna, and leg to promote differentiation. In the antenna, *ss* is consistently expressed, whereas in the eye, *ss* is variably expressed. My studies focus on *ss* expression in the leg, where it turns off in one region and turns on in a different region during development. To establish when *ss* turns off in the central region and later turns on in the anterior region of the leg imaginal disc, I conducted RNA FISH on wild-type flies at several timepoints between 98-120 hours after egg laying. Next, I will utilize the MS2-MCP system to track transcription in real time in the developing fly leg. Based on these data, I will generate a transcriptional bursting model, which creates a visualization of multiple bursts of transcripts over time. This model will shed light on how single molecules of RNA polymerase, transcription factors, and cofactors regulate gene activity at the single gene level. By assessing the bursting patterns of *ss* in the legs and comparing it to the antenna/eye, I will determine how the transcriptional dynamics of a single gene can impact its regulation of cell fate determination in different contexts.

457T **PARP-1 acts as a transcriptional rheostat during development** Gbolahan Bamgbose, Sarah Johnson, Alexei TulinBiomedical Sciences, University of North Dakota

PARP-1 is a transcriptional regulator that is essential for organismal development. Gene ablation of PARP-1/PARP-2 in mice, and PARP-1 in *Drosophila* causes developmental arrest. However, PARP-1's role as a regulator of gene expression during development remains to be elucidated. Here, using Drosophila, we interrogated the binding and transcriptional activity of PARP-1 in third-instar larvae, where developmental arrest of *Parp* mutants occurs. We found

two transcriptional changes mediated by PARP-1. First, PARP-1 preferrentialy occupies and dampens the expression of highly active metabolic genes, including glycolytic genes, in response to energy needs for transition to the pupal stage. Second, PARP-1 occupies and activates developmental genes, including a subset of bivalent genes. During larval-to-pupal transition in *Drosophila*, metabolic rate is reduced, likewise, the expression of metabolic genes as the animals prepare for a sessile and non-foraging pupal stage. In contrast, developmental genes, including bivalent genes bound and activated by PARP-1 are mainly expressed during embryogenesis and larval-to-pupal transition. As more studies continue to show the interdependence of metabolism and gene regulation in development and disease, we speculate that PARP-1 is a master transcriptional regulator tying these seemingly opposing processes together in metazoans. Hence, we propose that PARP-1 acts as a transcriptional rheostat to coordinate metabolic and gene expression programs during development.

458T **Exploring the Role of CLAMP's Prion-like Domain in Co-Transcriptional Splicing in Drosophila** Jasmine Shum, Mukulika Ray, Erica Larschan, Nicolas Fawzi, Julia ZaborowskyBrown University

Gene regulation is crucial to cellular function, driving cell fate decisions through transcription and RNA processing. Both transcription and splicing occur on chromatin and are coordinately regulated in a process known as co-transcriptional splicing. However, little is understood about its underlying mechanism. To investigate this mechanism, we studied a DNA/RNA binding protein Chromatin-Linked Adaptor for Male-Specific Lethal (MSL) proteins (CLAMP). CLAMP is known to contribute to male dosage compensation and sex-specific splicing, which involves ribonucleoprotein complexes called spliceosomes. The glycine-rich Prion-Like Domain (PrLD) within CLAMP, is essential for viability and contributes to sex-specific splicing. CLAMP also interacts with the Male Sex Lethal protein 2 (MSL2), an essential component of the MSL complex that coordinates dosage compensation in male Drosophila via the MSL2 binding domain (MSL2BD).

Biomolecular condensates are membraneless organelles that are formed by liquid-liquid phase separation, usually through RNA-protein interactions. Spliceosome complexes are reported to function as splicing condensates, but the mechanisms underlying their function are unclear. This project explores how RNA-protein complexes may drive spliceosome function via phase separation, and thus how different CLAMP domains contribute to context-specific function. It was hypothesized that the PrLD domain, known to facilitate phase separation properties, is essential for phase separation and thus splicing condensate formation while the MSL2 domain is essential only for dosage compensation. While previous individual-nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) data identified specific CLAMP-binding RNA motifs in male and female Drosophila, it is necessary to validate these motifs and understand whether the PrLD is essential for this binding. We generated CLAMP mutant plasmids with deletions of PrLD and MSL2BD (ΔMSL2BD) using PCR-based mutagenesis. Using electrophoretic mobility shift assays (EMSA), we assessed the RNA-protein interactions between CLAMP and RNA with these sex-specific motifs in vitro. In observing how different CLAMP mutants bind to RNA in cytoplasmic, chromatin, and nuclear fractions in male and female Drosophila, we elucidate the specific domains and RNA motifs that are important for CLAMP's regulation of co-transcriptional splicing through the biophysical properties of the relevant RNA-protein complexes.

459T **The *Mute* button: Turning down the volume of histone expression** Mark S Geisler[1], William F Marzluff[2], Robert J Duronio[11]Curriculum in Genetics and Molecular Biology, University of North Carolina - Chapel Hill, [2]Department of Biology, Integrative Program for Biological and Genome Sciences, Lineberger Comprehensive Cancer Center, University of North Carolina - Chapel Hill

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 the level of expression of RD-histone genes during S-phase is important for maintaining genomic integrity and normal cell cycle progression. Although the transcriptional activation of histone genes at the beginning of S phase has been extensively studied, much less is known about how histone transcription is terminated as cells exit S-phase or how transcription is modulated during S-phase to match histone gene expression with ongoing DNA replication. Prior research has shown that Mute, a component of the *Drosophila* Histone Locus Body (HLB), 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. We are interested in determining how Mute functions as a transcriptional repressor and its connection to the cell cycle regulation of the RD-histone genes. By fluorescent in situ hybridization to core histone mRNAs, we found that loss of Mute results in an increase in the number of cells expressing RD-histone genes in late-stage *Drosophila* embryos. Combining EdU labeling with FISH revealed that cells express RD-histones genes outside of S-phase, indicating

Mute couples expression of these genes to the cell cycle. We further showed Mute levels within the HLB change based on histone copy number, in contrast to other HLB factors whose recruitment to the HLB is independent of histone copy number. This suggests Mute may be acting as a modulator of histone transcription during S-phase. 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. These data lead us to hypothesize that Mute both restricts RD-histone expression to S-phase and modulates the level of transcription during S-phase to balance histone production with DNA replication. 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.

460T **Variable nuclear translocation kinetics differentially modulate gene expression during dorsal-ventral axis patterning** Samantha Fallacaro[1], Apratim Mukherjee[2], Mustafa A Mir[2,31]Developmental, Stem Cell, and Regenerative Biology, University of Pennsylvania, [2]Center for Computational and Genomic Medicine, Children's Hospital of Philadelphia, [3]Department of Cell and Developmental Biology, University of Pennsylvania

Transcription factors (TF) act as a bridge between signaling pathways and gene expression. Current models of signaling pathways often end with nuclear translocation of a specific transcription factor leading to transcription at its target genes. Previous work suggests that it is not the net change in the nuclear concentration of a TF but rather the kinetics of import that can lead to transcription of different target genes by the same TF. The molecular underpinnings of how dynamic nuclear translocation leads to differential transcriptional dynamics is poorly understood. I hypothesize that changes in nuclear translocation rate drive differential gene activation through modulation of protein microenvironments around target genes. To study the link between translocation kinetics and protein distributions, I use dorsoventral (DV) axis specification in early Drosophila embryogenesis as a model system. The morphogen Dorsal forms a nuclear concentration gradient along the DV axis specifying cell fates based on concentration-dependent gene expression. Nuclear import and export occurs along the DV axis at varying rates. While nuclear concentration has been quantified previously, how Dorsal's subnuclear organization and target gene transcription dynamics change with nuclear translocation rate is unknown. Here, I observe that the Dorsal nuclear translocation rate changes throughout nuclear cycles in the early embryo. Different translocation rates correlate with differences in the sub-nuclear organization of Dorsal resulting in varying size and number of high-local concentration protein hubs within the nucleus. I further investigate how translocation-kinetic dependent hubs influence transcription at endogenous Dorsal target genes. Additionally, I use single molecule tracking to assess if variations in translocation rates impact target search kinetics. Overall, I outline a potential mechanism where the translocation dynamics of a TF lead to alteration in their sub-nuclear distribution which modulates downstream gene expression. This mechanism will help resolve the mystery around how transcription factors decode upstream signaling dynamics to generate specific gene expression outputs which can be generalized to many systems involved in development and disease.

461T **Mechanisms of efficient transcription factor target search in *Drosophila* embryos** Alan P Boka[1,2,3], Puttachai Ratchasanmuang[4], Apratim Mukherjee[1,5], Joseph Zinski[1], Mustafa Mir[1,41]Cell and Developmental Biology, University of Pennsylvania Perelman School of Medicine, [2]Biochemistry and Molecular Biophysics Graduate Group, University of Pennsylvania Perelman School of Medicine, [3]Center for Computational Genomic Medicine, Children's Hospital of Philadelphia, [4]Center for Computational and Genomic Medicine, Children's Hospital of Philadelphia, [5]Children's Hospital of Philadelphia

Transcription factors exhibit highly specific binding to target regulatory regions of the genome. However, the molecular mechanisms underlying how transcription factors efficiently find their genomic targets in the crowded nuclear environment are not well understood. Recent live imaging studies have revealed that many transcription factors form high concentration "hubs" near target loci through homo- and heterotypic protein-protein interactions. It has been hypothesized that these interactions localize transcription factors to their target loci independent of their structured DNA binding domains, reducing search times and serving as a key determinant of specificity. Zelda is a pioneer transcription factor that serves as a maternal regulator of zygotic genome activation during early *Drosophila* embryogenesis. Zelda shows very high time-averaged occupancy at specific target *cis*-regulatory elements, however kinetic analysis of Zelda binding has shown that its residence time on chromatin is extremely transient (~5 sec). Zelda assembles into transient hubs, lasting 10's of seconds, during interphase, which have been hypothesized to increase Zelda's rate of chromatin binding. Here we use light-sheet microscopy, CUT&RUN, and single-molecule tracking to investigate the dynamics and binding specificity of Zelda in hubs within live embryos. We find that Zelda's zinc finger DNA binding domain is not required for hub formation or its localization at target regulatory elements. In addition, co-expression of wildtype

Zelda fully rescues the spatial organization of DNA-binding deficient Zelda, implicating homotypic protein-protein interactions in hub formation independent of DNA binding activity. In addition, the confined mobility of Zelda hubs is indistinguishable in the absence of specific DNA binding activity and single molecule imaging reveals the retained existence of an immobile chromatin-bound state in DNA-binding deficient Zelda. These data suggest that Zelda hubs facilitate transcription factor target search and specific chromatin binding through interactions independent of Zelda's DNA binding domain.

462T **Mechanisms of Fork head regulation of the salivary gland secretome** Dorian Jackson[1], Nathaniel Laughner[1], Dan Peng[2], Daniel Levings[3], Patrick Cahan[4], Matthew Slattery[3], Deborah Andrew[11]Cell Biology, Johns Hopkins University, [2]Biomedical Engineering, Johns Hopkins University, [3]Biomedical Sciences, University of Minnesota Medical School, [4]Cell Engineering, Johns Hopkins University

The FoxA family of winged-helix DNA binding transcription factors (TFs) are required for the development and survival of a wide variety of organs including the liver, lung, and pancreas. In these tissues, FoxA proteins serve as early transcriptional activators for tissue-specific gene expression. Interestingly, FoxA proteins have been shown to bind and open chromatin, suggesting that these proteins can act as the first ("pioneer") proteins to provide accessibility of other TFs to their tissue-specific downstream target genes during development. Whereas the FoxA family of TFs has three orthologues in mammals, Fkh is the sole FoxA family TF in Drosophila. Like its mammalian counterparts, Fkh is expressed in many tissues, including the foregut, hindgut, kidney, central nervous system (CNS), and the salivary gland (SG). One outstanding question about Fkh (and FoxA proteins in general) is how this singular "pioneer" protein can be expressed in so many distinct organs, yet still function to promote tissue-specific gene activities. Along with Fkh, two additional TFs – Sage and Senseless (Sens) – are activated in the early salivary gland primordia and are then continuously expressed throughout the developmental lifespan of this secretory organ. Previous studies in the salivary gland have revealed that Fkh works with Sage and Sens to activate expression of the SG secretome: SG cargo proteins and the enzymes that modify these proteins as they go through the secretory organelles. Here, we utilize tissue-specific ChIP-seq and scRNA-seq to identify all SG genes that are bound by Fkh, Sage, and Sens in the developing SG and to learn all of the SG genes whose expression is altered with loss of each of these TFs. These findings will allow us to uncover how Fkh coordinates its activity with that of Sage and Sens to control the SG secretome.

463T **Retinoblastoma paralogs employ both similar and distinct mechanisms of repression** Ana-Maria Raicu[1], Patricia Castanheira[2], David N Arnosti[21]Cell and Molecular Biology, Michigan State University, [2]Biochemistry and Molecular Biology, Michigan State University

CRISPR activation and interference (CRISPRa/i) have revolutionized gene expression studies, allowing us to precisely regulate genes through targeting activator and repressor domains across the genome. With CRISPRa/i, we can turn genes on and off with high precision and perform large-scale LOF and GOF screens in many systems. Yet, this modified CRISPR system is not often used for studying the mechanisms of gene regulation by transcription factors. Here, we have adapted the CRISPRi system for targeting transcriptional co-repressors to gene promoters to uncover their mechanisms of repression. We engineered dCas9 fusions to the Retinoblastoma family proteins (Rb), which are highly conserved co-repressors across Metazoa. In *D. melanogaster*, the Rb family consists of the Rbf1 and Rbf2 paralogs. Among arthropods, only Drosophila duplicated Rb and retained both genes. Vertebrates experienced their own duplication events leading to the expression of three Rb proteins. To uncover the significance of the multiplicity of the Rb family, when most eukaryotes have a single Rb that mediates all the conserved functions, we created dCas9-Rbf1 and dCas9-Rbf2 chimeras for tissue-specific expression in the fly. We targeted diverse gene promoters and uncovered paralog-specific mechanisms of repression. On some genes, both Rbf1 and Rbf2 are able to mediate potent repression, and this happens in a distance-dependent manner. The closer the Rb paralogs were brought to a gene's transcriptional start site, the greater their magnitude of repression, suggesting they impact the basal transcriptional machinery. We found examples in which Rbf2 was a more potent repressor, indicating the formation of different repression complexes. Notably, an Rbf1 mutant lacking the entire pocket domain, which was assumed to be necessary for function as a repressor, was just as good a repressor as the wild type protein. This suggests that the pocket may be required for recruitment to DNA but not for repression. Our novel adaptation of a well-established CRISPR tool has allowed us to probe mechanisms of repression *in vivo* in the fly and compare factors in the same contexts with great precision. This molecular analysis of promoter-specific regulation by Rb proteins will enhance our understanding of these conserved regulatory proteins in development and disease.

464T **Isoform expression and the post-transcriptional regulation of centrosome *Plp* mRNA** JUNNAN FANG, Weiyi (Rose) Tian, Dorothy LeritCell Biology, Emory University School of Medicine

The deregulation of centrosomes is associated with developmental disorders, including microcephaly, ciliopathy, and cancer. As major microtubule-organizing centers, centrosomes are composed of a pair of centrioles surrounded by pericentriolar material (PCM), a matrix of proteins required for microtubule nucleation. Also localizing to centrosomes are a small number of mRNAs. However, how and why RNAs localize to centrosomes remain critical unanswered questions. Conserved among the RNAs localizing to centrosomes is *pericentrin (PCNT)*-like protein (*Plp*). *Drosophila* PLP is a key component of the centrosome required for PCM scaffolding and microtubule organization. The disruption of *Plp* in *Drosophila* results in embryonic lethality, while the deregulation of *PCNT* in humans is associated with MOPD II and ciliary defects associated with Trisomy 21. To investigate mechanisms of *Plp* mRNA localization to centrosomes, we examined the contributions of two RNA-binding proteins during *Drosophila* embryogenesis. Our recent work shows oo18 RNA binding protein (Orb) interacts with *Plp* mRNA, contributes to *Plp* mRNA polyadenylation, and promotes PLP protein expression. Moreover, overexpression of full-length PLP can recover the PCM disorganization, cell division defects, and embryonic lethality caused by *orb* depletion, demonstrating *Plp* is an important downstream target of Orb. However, Orb is dispensable for robust *Plp* mRNA localization to centrosomes. To understand how *Plp* mRNA localizes to centrosomes, we investigated another multifunctional RNA binding protein, FMRP, the product of the fragile X mental retardation 1 gene (*Fmr1*). Our preliminary data indicate FMRP interacts with *Plp* mRNA and promotes its localization to centrosomes. These data support the idea that different RNA binding proteins coordinate centrosomal mRNA localization and translation. In addition, we observed the *Plp* coding region is required and sufficient for mRNA localization, and we uncovered a critical region for its localization at centrosomes. Consistent with a model requiring active polysome transport, treatment with translation inhibitors impairs *Plp* mRNA localization. *Drosophila Plp* gene encodes 12 RNA isoforms, and our recent work show that different *Plp* RNA isoforms are differentially expressed in embryos, we will present our efforts to investigate the isoform-specific RNA localization. This work lends mechanistic insights into RNA localization to centrosomes, which may be involved in the etiology of human diseases.

465T **Bicoid concentration differentially regulates the transcriptional onset of targets genes** Eleanor Degen, Shelby BlytheMolecular Biosciences, Northwestern University

When the concentration of a transcription factor varies across a tissue, nuclei convert this input into differential transcriptional outputs. In the early Drosophila embryo, the exponentially distributed transcription factor Bicoid regulates a network of patterning genes across the anterior-posterior axis. Bicoid directly influences the spatiotemporal expression patterns of its target genes through concentration-sensitive binding to enhancers, and indirectly through regulatory feedback within the patterning gene network. While mutant studies have demonstrated that gene expression domains rely on Bicoid, we do not fully understand the regulatory relationship between Bicoid concentration and transcriptional output across the entire network. By live-imaging MS2-MCP transcriptional reporters, we have found evidence suggesting that concentration-sensitive Bicoid binding to enhancers mediates the speed of transcriptional onset in anterior domains following mitosis. The delay after mitosis that precedes transcription driven by concentration-sensitive Bicoid binding in the anterior negatively correlates with Bicoid concentration. Conversely, a reporter for an enhancer that regulates a posterior Bicoid-dependent gene expression domain does not display transcriptional onset times that change with Bicoid concentration. The polymerase loading rates of all tested patterning gene reporters also do not change with Bicoid concentration. We therefore propose that feedback from the patterning gene regulatory network acts on the polymerase loading process to dampen Bicoid target gene expression at the boundaries of their domains. These findings allow us to begin to decouple the direct effects of Bicoid binding from the indirect effects of regulatory network feedback on the transcriptional process.

466F **Sequence reliance of a context-dependent transcription factor** Lauren Hodkinson[1], Julia Gross[2,3], Leila Rieder[11]Department of Biology, Emory University, [2]National Institute of Allergy and Infectious Disease, [3]Emory University

Despite binding similar *cis* elements, transcription factors can perform context-dependent functions at different locations across the genome. To explore this phenomenon, we broadly aim to understand how transcription factors integrate *cis* sequence and genomic context to function uniquely at different loci, which is critical for development and disease. One example of a context-specific transcription factor 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 transcription factors. 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), which regulates expression of the replication-dependent histone genes. Here we investigate how the function of CLAMP at the histone locus is impacted by the origin of its *cis* binding elements. CLAMP binds a long GA-repeat element in the bidirectional promoter of histone genes 3 and 4 (*H3H4p*). We engineered flies to carry a transgenic histone array in which we replaced the natural GA-

repeating *cis* element in the *H3H4p* with CLAMP-recruiting GA-rich elements from the X chromosome. We assessed how X-linked *cis* elements impact HLB formation by staining third instar larval polytene chromosomes with antibodies specific to a core HLB protein as well as an X chromosome specific factor. When we replaced the *H3H4p* 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, the transgene retained both the ability to recruit the HLB factor or the X-chromosome factor to the transgene. Our observations indicate that both sequence and context dictate CLAMP function. In the future, we will assess how the different GA-rich *cis* elements impact histone gene transcription using qRT-PCR to further evaluate locus function.

467F **Regulation of Chiffon products in *Drosophila* embryogenesis** Anik Paul[1], Mohd Saleem Dar[2], Vikki M. Weake[21]Purdue University, [2]Biochemistry, Purdue University

The Cdc7 kinase along with its regulatory partner Dbf4 (Dumbbell-forming 4) signals to initiate DNA replication in organisms ranging from fungi to mammals. In *Drosophila*, the sole Dbf4 ortholog is Chiffon, which was named because *chiffon* mutant females lay eggs with thin, fragile eggshells. We previously showed that the Chiffon N terminal region interacts with Cdc7 to form the DDK (Dbf4 Dependent Kinase) complex that is essential for both chorion gene amplification in follicle cells and rapid DNA replication during the maternally controlled early nuclear divisions in embryogenesis. In flies, Chiffon possesses a long C-terminal extension that interacts with Gcn5 to form the Chiffon histone acetyltransferase (CHAT) complex. CHAT acetylates histone H3 *in vivo* and loss of CHAT activity causes widespread changes in gene expression in embryos and lethality. The C-terminal extension of Chiffon responsible for CHAT formation is only present in insects, and preliminary studies suggest this complex might be restricted to the Diptera. Intriguingly, we showed that the CHAT activity of Chiffon rather than its ancestral DDK function is essential for viability in flies. Our preliminary genetic data suggest that the DDK and CHAT functions of Chiffon are independent, and that *chiffon* encodes distinct N- and C-terminal products despite the entire coding region being contained within one large ~5kb exon. Here, we will present data describing the mechanisms involved in Chiffon regulation that control the production of distinct DDK and CHAT complexes during early embryogenesis.

468F **Investigating the *cis*-regulatory elements controlling a stochastic cell fate choice in the *Drosophila* eye** Yaniris Molina, Lukas Voortman, Elizabeth Urban, Alison Ordway, Robert JohnstonBiology, Johns Hopkins University

Stochastic gene regulatory mechanisms diversify cell types and generate random patterns in tissues. Disruption of these mechanisms can lead to human diseases, including vision impairment and lymphoma. How gene expression is stochastically regulated during development remains poorly understood. Here we aim to study stochastic gene expression in the *Drosophila melanogaster* (fruit fly) eye. The fly eye contains 800 unit eyes, each composed of eight photoreceptor cells (R1-R8). The R7 photoreceptor has two distinct fates, defined by expression of either Rhodopsin 3 (Rh3) or Rhodopsin 4 (Rh4). This fate decision is stochastic and Rh3- and Rh4-expressing R7s are randomly patterned across the eye. This fate decision is regulated by the stochastic on/off expression of the transcription factor Spineless (Ss): Ss on R7s express Rh4 and Ss off R7s express Rh3. To address how the stochastic expression of *ss* is controlled, we are using CRISPR-Cas9 technology to mutate enhancers that regulate *ss* expression in the eye. The *early enhancer* promotes *ss* expression in R7 precursor cells and the *late enhancer* drives expression in terminal R7s. We are generating deletions within the endogenous enhancers that retain local chromatin environment inputs. By targeting these enhancers, we expect to identify putative transcription factor binding sites. For our genome engineering strategy, we are using two guide RNAs that flank the region of interest in the *ss* locus. To generate a defined deletion, we are using a plasmid-based donor repair template with a selectable marker. The guide RNAs and homology directed repair (HDR) donor plasmid will be microinjected into transgenic fly lines expressing Cas9 under a germline promoter (vasa > Cas9). The genomic modifications will be validated using Sanger DNA sequencing. We will examine mutants for changes in Rh3:Rh4 expression. We predict that sub-regions of the enhancers will be required for the expression of *ss*. Our findings will provide mechanistic insight into the regulatory DNA elements that govern stochastic Ss expression during development.

469F **Investigating Vasa's role in Translational Regulation of Oskar Protein** Austin Chiappetta, Siran Tian, Tatjana TrcekBiology, Johns Hopkins University

Germ granules are germline biomolecular condensates which are important for post-transcriptional regulation during gametogenesis and early development. In early *Drosophila* embryos, germ granules have been associated with the specification of primordial germ cells and establishing proper embryo body patterning. *Drosophila* germ granules are

nucleated by the protein Oskar. Oskar protein is encoded by *oskar* mRNA, which localizes to the posterior tip of the developing oocyte. There, *oskar* mRNA is translated to produce two protein isoforms, Long Oskar and Short Oskar, which are the result of alternative translation initiation site usage. The two Oskar isoforms have distinct functions: Short Oskar nucleates germ granules, whereas Long Oskar anchors the germ plasm at the posterior.

The germline-specific DEAD-box RNA helicase Vasa is critical for accumulation of Oskar. Without affecting Oskar protein stability, Vasa appears to specifically promote translation of Short Oskar, having no effect on expression of Long Oskar. How Vasa achieves this regulation of Oskar is unclear. To gain insight into the mechanism of Vasa's regulation of *oskar* mRNA translation, we employed DMS-MaPseq to measure Vasa's effect on *oskar* mRNA secondary structure. Intriguingly, we observe that in the presence of Vasa, the Short Oskar start codon is structurally exposed whereas in the absence of Vasa it is partially embedded within a hairpin. We are now testing whether this structural change is sufficient to alter translation start site usage and whether Vasa's effect on *oskar* translation is direct or indirect. To further investigate the mechanism of Vasa's activity, a fluorescent reporter of Oskar translation initiation was developed and used in combination with mutations affecting Vasa's RNA-binding and helicase activities to measure their effects on Vasa's ability to regulate Oskar. In addition, sequence mutagenesis of the reporter is being used to map *oskar* mRNA sequences necessary for regulation by Vasa. This research not only sheds light on Vasa's regulation of *oskar*, but also on how helicases can influence translation initiation more generally. This work was supported by the R35GM142737 NIGMS grant awarded to TT.

470F **FSH Plays a Critical Role in Zelda Mediated Zygotic Genome Transactivation During Early Embryogenesis in *Drosophila*** Xiao-yong Li[1], Michael B Eisen[1,21]Howard Hughes Medical Institute, University of California, Berkeley, CA, [2]Department of Molecular and Cell Biology, Department of Integrative Biology, QB3 Institute, University of California, Berkeley

The maternal factor Zelda binds broadly to zygotic enhancers and critical for transactivation of large number of early zygotic genes during maternal to zygotic transition in *Drosophila*. It represents a special class of factors which function primarily by facilitating access of other transcription factors to enhancers to potentiate enhancer function, which most likely requires it to interact with certain cofactors. To identify such factors, we have employed chromatin immunoprecipitation coupled with mass spectrometry. Using an anti-Zelda antibody for the pull-down, we saw significant enrichment of some common transcription factors, as well as various factors that play important roles in modulating chromatin structures. In particular, the bromodomain containing factor FSH encoded by the *fs(1)h* gene that has previously been implicated in enhancer activity is one of the most enriched factors, and was chosen for further investigation. Based on immunofluorescence analysis, we found FSH forms clusters in the early embryonic nuclei, which is temporally correlated with zygotic genome activation. In *zld*[CH1] mutant embryos, the large FSH clusters mostly disappeared in the nuclei of early embryos, in correlation with a dramatic decrease in the number of histone H3 K18 acetylation foci[CH2] . Further we found RNAi knock down of *fs(1)h* significantly decreased transcription of *tld* and *sala*, [CH3] while having modest to no effect on the transcription of the gap genes *hb* and *btd*, which is consistent with their varying dependence on Zelda activity. These findings together suggest FSH is involved in Zld function and plays an important role in early zygotic gene activation during early embryogenesis in *Drosophila*.

[CH1]I would italicize here to indicate it's the gene name, since you're talking about a mutant, but that might be mostly stylistic

[CH2]From the way this is written, it's unclear if H3K18 acetylation is something that you looked at yourself, or if you're comparing your FSH results to published data.

[CH3]It would be good to have a one or two word description of what these genes are and why they are relevant

471F **A hierarchy influencing gene expression** Siddhant Kalra, Stephen Lanno, Lupita Sanchez, Joseph CoolonBiology, Wesleyan University

Decades of genetic research have shown that there are multiple sources which are responsible for variations in traits of an organism. Examples of some of these sources are genotypic, environmental, developmental and transgenerational effects on trait outcomes. While a lot of studies have demonstrated a multitude

of effects of each source of variation in a wide variety of organisms, the relative contribution of these sources of variation remains largely unknown especially for transgenerational effects. Here we compared the role of each of these factors on genome wide gene expression in two Drosophila species (D. simulans and D. sechellia). We subjected them in control and octanoic acid environments and studied the changes in their gene expression due to their current and previous generation environmental exposure along with contribution of difference in species and developmental stages.

472F **The complexity of genomic DNA methylation influences sex-specific functions in environmental changes in cactophilic *Drosophila*** Adriano S. Santos[1], Ester S. Ramos[1], Fábio M. Sene[1], Maura H. Manfrin[2,31]Genetics, Ribeirão Preto Medical School, University of São Paulo, FMRP-USP, [2]Biology, Faculty of Philosophy, Sciences, and Letters of Ribeirão Preto, University of São Paulo, FFCLRP-USP, [3]Genetics, FMRP, University of São Paulo

Introduction: DNA methylation with 5-methyl-cytosine (5mC) has been described in several eukaryotic genomes, with roles in regulating genes, phenotypes, environmental stresses, and influencing phenotypic plasticity responses. The 5mC can be associated with phenotypic plasticity, found in viability, development, and morphological structures when *Drosophila gouveai* uses breeding sites in *Pilosocereus machrisii* or *Cereus hildmannianus* cacti. **Our objective** was to analyze changes at multiple methylated *loci* in DNA (CpC and CpG sites) and to identify the molecular targets involved when organisms develop at different ecological sites, in *D. gouveai*. **Material and Methods:** Biological samples of *D. gouveai* reared separately in different environments: *P. machrisis* tissues (I), *C. hildmmanianus* tissues (II) and laboratory standard (control) (III); dissection of gonads tissues of virgin adult flies (female and male). We isolated DNA from samples and performed Methylation-Sensitive Amplification Polymorphism (MSAP) assays to evaluate genome-wide, verified with a Polyacrylamide gel, when organisms grow in different environments. We sequenced regions with methylation differences and evaluated the functions with bioinformatics tools (Blast, GO, KEGG). **Results:** We obtained 295 *loci* in female reproductive tissues and another 200 *loci* in male flies, with 12 MSAP markers. Complexity in DNA methylation was found concerning the sex and breeding site of the flies. Multiple methylation-sensitive *loci* in female flies that grew on laboratory standard (82 *loci*), others on *C. hildmannianus* tissues (65 *loci*), and with *P. machrisii* (52 *loci*), with statistical in One-way ANOVA (F=2.6618; P-value=0.102). Changes in the methylation pattern in male flies reared on standard medium (55 *loci*), others in *C. hildmannianus* tissues (54 *loci*) (external methylation pattern; P=0.03), and with *P. machrisii* (42 *loci*). Differential DNA methylation targets are related to different biological processes and functions: *Piezo* gene with mechanosensory role and recognition of oviposition substrate in fly females in *C. hildmannianus*, *eEF1alpha1* gene with activity in protein synthesis and nucleus-cytoplasm communication (males and females in *P. machrisii*), Galileo transposable element in fly males in *C. hildmannianus*. **Conclusion:** DNA methylation heterogeneity is related to sex-specific differences and changes in breeding sites, with implications for molecular physiological and metabolic pathways in *D. gouveai*.

473F **Characterizing the Role of METTL3 During *Drosophila* Embryogenesis.** Savannah L Barton, Antonio L RockwellSusquehanna University

The methylation of m6A is the most abundant internal modification found in eukaryotic mRNA. This modification has many roles in mRNA metabolism. METTL3, the enzyme that adds m^6A is evolutionarily conserved. METTL3 is required for various biological processes including embryogenesis. Data collected from several studies suggest METTL3 is essential for viability in murine and plants. In *Drosophila*, METTL3 mutants have reduced viability or fail to eclose entirely. Despite this work being conducted over the past several years, a mechanism explaining METTL3's essentiality has not been fully elucidated. Our lab's previous work suggests METTL3 regulates an essential gene profilin (chic) in the testes. However, that work examined METTL3 in a non-essential process spermatogenesis. Our lab is currently working to characterize METTL3 in an essential process embryogenesis. The aim is to determine a mechanism that explains METTL3's impact on viability. Using immunostaining, we have characterized METTL3 and m^6A localization pattens throughout stages of embryogenesis with highest levels occurring between embryonic stage 9 the start of CNS development and stage 15. Depletion of functional METTL3 in mutant stocks results in embryonic arrest before larval stage 1 is reached. Mutants appear to have abnormal localization pattern of CNS marker Prospero. Additionally, f-actin patterns appear to be irregular in our mutants compared to controls. Preliminary RT-qPCR data suggests that chic which is needed to regulate f-actin is being downregulated in mutants. The data we have collected to date leads us to hypothesize that METTL3 is regulating chic within the *Drosophila* embryo. Profilin is essential and evolutionarily conserved similar to METTL3. Our findings may potentially explain METTL3 essentiality in some multicellular organism development.

474F **The conserved homeodomain transcription factor Hmx rewires the Hippo pathway to specify and maintain blue-sensitive photoreceptor fate** Joseph Bunker[1], Brooke Cayting[2], Sydney Bailey[2], Mhamed Bashir[2], Jens Rister[21]Biology, UMass Boston, [2]Biology, Umass Boston

Signaling pathways are repurposed in different developmental contexts to elicit a variety of cellular responses. For instance, the Hippo pathway was initially discovered as a tumor suppressor pathway in mitotically active tissue. However, in post-mitotic photoreceptors (PRs), the pathway is repurposed to regulate a binary fate decision: pathway activation promotes the expression of the green-sensitive pigment Rh6 (Hippo ON/Rh6 fate), and inactivation of the pathway promotes blue-sensitive Rh5 (Hippo OFF/Rh5 fate). The nexus of the pathway, the Warts (Wts) kinase, represses the transcriptional co-activator Yorkie (Yki) through phosphorylation. In dividing tissues, unphosphorylated Yki activates growth-promoting genes but also promotes Hippo pathway activity; thus, Yki activity is controlled via negative feedback. In post-mitotic PRs, Yki activates *Rh5* and represses *Rh6*, and contrary to its role in growth, Yki represses Hippo pathway activity by repressing *wts*. How Yki represses *Rh6* and *wts* remains poorly understood.

We identified the homeodomain transcription factor Hmx as a novel regulator of the Hippo pathway. *Hmx* is exclusively expressed in Hippo OFF/Rh5 PRs and Yki is both necessary and sufficient for *Hmx* expression. Loss of *Hmx*, either through RNAi-mediated knockdown or generation of *Hmx* null mutant clones, causes a loss of Hippo OFF/Rh5 PRs. Conversely, overexpression of *Hmx* causes a gain of Hippo OFF/Rh5 PRs, strongly suggesting that Hmx is necessary and sufficient for Hippo OFF/Rh5 fate. Moreover, through epistasis analyses, we show that Hmx acts downstream of Yki to repress Rh6. We identified a conserved Hmx motif in the *Rh6* promoter and show that Hmx represses this promoter *in vitro* by antagonizing the homeodomain transcription factor Pph13. Finally, we also show that Hmx is necessary and sufficient to repress a *wts*-GFP transcriptional reporter *in vivo*. Taken together, *Hmx* is activated by Yki to repress both *Rh6* and *wts*, suggesting that Hmx rewires the Hippo pathway to promote Hippo OFF/Rh5 fate in post-mitotic color PRs.

475F **ETS-domain transcriptional regulators Pnt and Yan regulate spatiotemporal expression of matrix metalloproteinase 2 in *Drosophila* ovary** Baosheng Zeng[1], Ekaterina Skaritanov[1], Elizabeth M Knapp[2], Jianjun Sun[11]Physiology and Neurobiology, University of Connecticut, [2]Physiology and Neurobiology, University fo Connecticut

Precise regulation of matrix metalloproteinase expression and activation is critical for tissue remodeling and homeostasis; however, the underlying mechanisms are incompletely understood. Our previous work showed that matrix metalloproteinase 2 (MMP2) is specifically expressed in posterior follicle cells of a mature egg chamber (stage 14), which is crucial for the breakdown of the follicle wall and the liberation of the encapsulated mature oocyte during ovulation, a process highly conserved from flies to mammals. It remains unknown what signaling mechanisms regulate this precise spatiotemporal expression of MMP2 in posterior stage-14 follicle cells. Here we identify a novel role for the ETS transcriptional activator Pointed (Pnt) and its endogenous repressor Yan in regulating MMP2 expression and follicle rupture. Pnt is localized to posterior follicle cells throughout the late oogenesis and its expression domain overlaps with MMP2 expression in stage-14 egg chambers. Genetic gain- and loss-of-function analysis demonstrated that *pnt* is both required and sufficient for MMP2 expression in follicle cells. In addition, we found that its repressor Yan is rapidly downregulated at stage 14, which is critical for the alleviation of transcriptional suppression and MMP2 expression. Furthermore, we identified a ~1.1 kb upstream cis-regulatory element from *mmp2* gent that is responsible for the spatiotemporal expression of MMP2 and contains multiple *pnt/yan* binding motifs. Currently, we are carrying out point mutation analysis to demonstrate the direct regulation of *mmp2* by Pnt and Yan. Our data reveal new insights into the transcriptional regulation of *mmp2* expression in *Drosophila* ovary, which could be applied to other biological systems as well.

476F **Dorsal/NF-κB exhibits a dorsal-to-ventral mobility gradient in the *Drosophila* embryo** Etika Goyal[1], Hadel Y Al Asafen[2], Natalie M Clark[2], Thomas Jacobsen[2], Rosangela Sozzani[2], Gregory Reeves[11]Texas A&M University, [2]North Carolina State University

Morphogen-mediated patterning is a highly dynamic developmental process. To obtain an accurate understanding of morphogen gradients, biophysical parameters such as protein diffusivities must be quantified *in vivo*. The dorsal-ventral (DV) patterning of early *Drosophila* embryos by the NF-κB homolog Dorsal (Dl) is an excellent system for understanding morphogen gradient formation. Dl gradient formation is controlled by the inhibitor Cactus/IκB (Cact), which regulates the nuclear import and diffusion of Dl protein. However, quantitative measurements of spatiotemporal Dl movement are currently lacking. Here, we use scanning fluorescence correlation spectroscopy to quantify the mobility of Dl. We find that the diffusivity of Dl varies along the DV axis, with lowest diffusivities on the ventral side, and the DV asymmetry

in diffusivity is exclusive to the nuclei. Moreover, we also observe that nuclear export rates are lower in the ventral and lateral regions of the embryo. Both cross correlation spectroscopy measurements and a computational model of Dl/DNA binding suggest that DNA binding of Dl, which is more prevalent on the ventral side of the embryo, is correlated to a lower diffusivity and nuclear export rate. We propose that the variation in Dl/DNA binding along the DV axis is dependent on Cact binding Dl, which prevents Dl from binding DNA in dorsal and lateral regions of the embryo. Thus, our results highlight the complexity of morphogen gradient dynamics and the need for quantitative measurements of biophysical interactions in such systems.

477F **K-Gut Project Creates a Database of High-resolution Gut-expression Images of GAL4 Transgenes Derived from *Drosophila* Gut-expressed Genes** Seung Yeon Lim[1], Hyejin You[2], Jinhyeong Lee[3], Sung-Eun Yoon[4], Jae Young Kwon[1], Won-Jae Lee[2], Young-Joon Kim[5], Greg S B Suh[31]Sungkyunkwan University, [2]Seoul National University and National Creative Research Initiative Center for hologenomics, [3]Korea Advanced Institute of Science and Technology, [4]Korea Drosophila Resource Center, [5]Gwangju Institute of Science and Technology

The gastrointestinal tract of adult *Drosophila* is used as a model system to study mechanisms regulating key biological processes, including food digestion and nutrients absorption, immune defenses, interactions with the microbiome, nutrient sensing, and inter-organ communications, particularly through the gut-brain axis. Because the gastrointestinal tract is composed of many different cell types and regions, molecular genetic tools that target and manipulate gene expression for specific cell types and regions are essential for these studies. To address this unmet need, Korea *Drosophila* Resource Center (KDRC) and three research institutes in Korea have teamed up to launch the K-Gut initiative, which aims to develop a comprehensive collection of GAL4 lines and split GAL4 lines that can be used to easily manipulate individual cells in the gastrointestinal tract of adult *Drosophila*. Based on RNA sequencing data, K-Gut team collected about 900 GAL4 strains derived from genes expressed in the gut and investigated the expression patterns by cell type and region in the gastrointestinal tract. Through the K-GUT initiative, we have identified the expression of hundreds of GAL4 lines that mark enteroendocrine cells, enterocytes, enteroblasts, stem cells and enteric neurons, which have so far made available to scientists worldwide through the KDRC website, http://kdrc.kr/index.php. The K-GUT initiative goes further to generate split-GAL4 lines to display and manipulate subsets of specific cell types in the gut.

478F **Identifying the impact of H3K4 methylation on the circadian clock in the *D. melanogaster* eye** Gaoya Meng, Vikki WeakeBiochemistry, Purdue University

To synchronize with environmental stimuli, predominantly light, the circadian clock directs the physiological and behavioral cycles with a periodicity of approximately 24 hours. The circadian clock is progressively dysregulated during aging, correlating with the development of many neurodegenerative diseases. Circadian disruption in the eye affects retinal development and accelerates photoreceptor degeneration during aging, which implies a protective effect of the functional circadian clock on photoreceptor neurons. At the core of the molecular clock, Clock (CLK) and Cycle (CYC), a pair of transcription activators, regulate the rhythmic transcription of output genes, including a substantial fraction of phototransduction genes. In the aging photoreceptors, CLK:CYC exhibit differential activity while the mechanisms that contribute to this change and its impact on downstream gene expression are poorly understood. H3K4me3 is an active transcription mark that is present at promoters and contributes to chromatin accessibility. Our research shows that the loss of H3K4me3 has a significant effect on circadian gene expression in photoreceptors. By profiling nuclear transcriptome throughout 24h day, we identified in the absence of H3K4me3, 9% expressed genes in photoreceptors lose rhythmic transcription, many of which are enriched in cognition and mitochondrial gene expression. Surprisingly, 5% of genes gain rhythmic transcription, including stress response genes. In addition, all of the core clock genes showed decreased expression amplitude, indicating H3K4me3 elevates clock-controlled transcription activation. Given that global decrease of H3K4me3 is observed in the aging eye, our study favors the scenario where decreased H3K4me3 levels during aging reduces clock-controlled transcription. Our future work will continue to elucidate the distinct mechanisms underlying the changes in rhythmic expression patterns in the absence of H3K4me3.

479F **Comprehending testes-specific gene regulation in *Drosophila melanogaster*** Saurabh Chaudhary[1], Sabrina Williams[2], Shrinivas Nivrutti Dighe[2], Katia Jindrich[3], Helen White-Cooper[21]School of Biosciences, Cardiff University, [2]Cardiff University, [3]University of Oxford

As *Drosophila* male germline cells differentiate, high transcriptional dynamics ensure activation of the expression of many genes in spermatocytes. The transcriptome complexity s well known; however, the underpinning molecular mechanism regulating the coordinated expression of thousands of genes in *Drosophila* testes remains largely

undetermined. Testis Meiotic Arrest Complex (tMAC) is required to ensure high levels of transcription of many genes, specifically in spermatocytes. This complex contains protein subunits with DNA binding domains, as well as additional proteins not predicted to bind DNA. In this study, we dissect how tMAC interacts with target promoters by identifying *in vitro* binding motifs using high-throughput SELEX (HT-SELEX) sequencing. After bioinformatics analysis, DNA-binding motifs identified after 2, 4 and 6 rounds of SELEX were considered confident motifs. Therefore, those motifs were used further for their presence at the promoter regions of the expressed genes in the previously designed several promoter-reporter constructs such as *aly* (one of tMAC subunits) dependent (*aly* mutant) and *aly* independent (wild type). The occurrence of the motifs suggests high potential binding sites in the promoter regions of *aly* mutant as compared to the wild type. For *in vivo* analysis, we developed GFP-tagged *Drosophila* lines using CRISPR-Cas9 to perform chromatin immunoprecipitation sequencing (ChIP-Seq). By integrating the *in vivo* (ChIP-Seq) data with the *in vitro* (HT-SELEX) binding site motif information, we will be able to determine which DNA-binding proteins contribute to the binding of tMAC to any specific promoter sequence.

480F **Regulation of transcriptional dynamics in *Drosophila* embryonic heart development** Amanda Hill, Suzy Hur, Isaak TarampoulousThe University of Chicago

During organ development, undifferentiated stem cells undergo a series of fate decisions as they divide to produce a wide array of tissue-specific cell types. In order to achieve proper tissue development and function, cell fate decisions must be precisely and reliably regulated. Extensive efforts have identified short and long-range signaling strategies that control the spatial expression of fate-determining genes. However, we currently have a poor quantitative understanding of how the dynamic expression of gene products is appropriately tuned to produce sufficient mRNA levels for particular cell types.

In the stage 11 *Drosophila* embryo, expression of the gene *even-skipped* (*eve*) becomes progressively refined from a cluster of approximately 12 initially Eve-positive mesodermal cells to a single cardiac precursor cell. Previous work has characterized the role of two antagonistic transcription factors that are crucial to the precision of this developmental process; a general transcriptional activator, Pointed (Pnt), promotes Eve expression, while a transcriptional repressor, Yan, represses it. How Pnt and Yan protein concentrations are fine-tuned to regulate *eve* mRNA expression dynamics during cardiac precursor specification remains unknown.

Therefore, investigating *eve* mRNA dynamics in embryonic heart development has the potential to reveal novel insights into how Eve expression is stably turned on or off in subsets of cells to reliably specify cardiac precursor cells. We are combining single molecule FISH and protein immunostaining to understand the input-output relationships between Yan and Pnt protein concentrations and *eve* transcriptional dynamics. By examining wildtype and various perturbation conditions to tune Yan and Pnt interactions with the mesodermal *eve* enhancer, we will present how the fine-scale transcriptional dynamics of a developmental gene are precisely controlled by combined transcription factor inputs during cell fate specification.

481F **Investigating how germline sexual identity controls sex-specific gene expression** Harrison A Curnutte[1], Adam Winter[1], Caitlin Pozmanter[1], Mark Van Doren[21]Johns Hopkins University, [2]Johns University

In nature, different sexes of a sexually reproducing species exhibit sexual dimorphism, or differences in morphology and behavior between sexes. One striking morphological difference between the sexes are the gonads, the testis and ovary, which produce the sperm and egg necessary for sexual reproduction and perpetuation of the species. In order to develop proper gametes, both the germ cells and the somatic cells of the gonad must decide their sexual identity. While sex determination is well characterized in the somatic gonad of *Drosophila*, little is known about how the germ cells determine their sex. The RNA binding protein Sex lethal (Sxl) has been shown to be a master regulator of somatic sex determination, where it can act in the nucleus as a splicing factor or in the cytoplasm to repress translation. Sxl is also important in the germline, where it is both necessary and sufficient for female germline identity, making it a central regulator for germline sex. Unlike the soma, sex determination downstream of Sxl is not well characterized in the germline, making it an intriguing subject of study.

A key aspect of sex determination is the control of sex-specific gene expression, about which little is known in the germline. We will examine expression of Tdrd5l (Tudor domain-containing protein 5-like), which is expressed in a male-specific manner in the early germline and is important for male germline sexual identity. Tdrd5l RNA, but not protein, is initially expressed in the germline of both sexes, providing a model for how germ cell identity leads to germ cell-specific transcription. Expression of Tdrd5l is subsequently repressed in female germ cells by Sex lethal (Sxl). Deletion of putative

Sxl binding sites (polyU tracts) in an intronic region and the 3'UTR of the Tdrd5l primary transcript causes derepression of Tdrd5l protein expression (Primus et al., 2019), indicating that part of the sex-specific regulation is post-transcriptional. In addition, loss of Sxl function in female germ cells causes a 16-fold increase in Tdrd5l mRNA levels (Primus et al., 2019), indicating that either the post-transcriptional regulation acts partly via mRNA stability, or that sex-specific transcriptional regulation is also involved. Thus, a study of Tdrd5l regulation will provide insight into the multiple levels by which sex-specific gene expression is regulated in the germline.

482F **Shining a Light on the Design Principles of Developmental Shadow Enhancers** Jillian Ness, Zeba WunderlichBiology, Boston University

Many developmental genes have been shown to be regulated by enhancers that drive expression in the same or overlapping spatiotemporal patterns. These sets of enhancers are termed shadow enhancers. Even though shadow enhancers are a ubiquitous feature of developmental gene regulatory networks (GRNs) in mammals, insects, and plants,there is still much to learn about their purpose and functionality in development. The aim of this study is to elaborate on the design principles of developmental shadow enhancers using a series of synthetic biology-based experiments.

The *Drosophila melanogaster* gap gene loci are known to be a useful system for probing the design principles of developmental shadow enhancers. These shadow enhancers need to funnel noisy upstream TF inputs to drive robust gene expression of gap genes for early-embryo body patterning. Studies of the *Kruppel* (*Kr*) gap gene locus show a shadow enhancer set can have *different* TF activators, rather than identical ones. This distinct regulatory logic of shadow enhancers within a GRN is termed a 'separation of inputs.' The separation of inputs found in *Kr's* shadow enhancers buffers expression levels against fluctuations in upstream TF levels.

In this study, the developmental *nubbin* (*nub*) and *giant* (*gt*) gap gene shadow enhancer pairs are investigated. Unlike *Kr*, these genes have shadow pairs with an **overlap** in TF input profiles. We monitor the activity of these shadow enhancer sets using an innovative technique that measures live transcriptional dynamics over time using an mRNA fluorescence reporter system. The measured transcriptional output over time, driven by the enhancer configurations, gives insight into the noise and correlated activity of the shadow pairs in the system. Ultimately, with this project, we unpack the design principles of shadow enhancers to understand their function and prevalence across organisms during development.

483F **Gene expression regulation by the Bin3 noncoding RNA methyltransferase through 7SK snRNP-dependent and -independent mechanisms plays a conserved role in development** Ryan J Palumbo, Steven D HanesBiochemistry & Molecular Biology, SUNY Upstate Medical University

Promoter proximal pausing of RNA Pol II is a regulated mechanism that is essential for development and cellular homeostasis. Pausing is released by the activity of the P-TEFb heterodimer, comprising the kinase Cdk9, and CycT. P-TEFb is regulated by sequestration into the 7SK snRNP. The 7SK ncRNA is a scaffold for the proteins MePCE and Larp7, which protect the ends of 7SK, and Hexim, which—after binding to 7SK—can then bind and represses P-TEFb. MePCE is an RNA methyltransferase (MTase) that binds to and methylates the 5' end of 7SK, producing a cap structure that is alleged to prevent 5'→3' degradation and ensure 7SK stability. Importantly, *MePCE* haploinsufficiency causes a neuromuscular disorder characterized by 7SK instability, hyper-activated P-TEFb, and altered gene expression; however, little else is known about the role of MePCE in transcription regulation to promote normal development. *Drosophila* Bin3 is the founding member of the Bin3/Bmc1/MePCE family of RNA MTases. Previously, we found that Bin3 regulates *caudal* translation during embryogenesis by interacting with Bicoid, an insect-specific protein. Here, we studied the role of Bin3 specifically in transcriptional regulation to promote normal fly physiology, a context that is more relevant to human health. We found that Bin3 has a conserved role in promoting neuromuscular development by repressing P-TEFb, as hyper-active P-TEFb in *bin3* flies caused defects in climbing and wing posture that were rescued by genetically reducing *CycT* levels. We also found that Bin3 functions specifically in somatic cells to promote female fecundity. Strikingly, we found that Bin3 catalytic activity is dispensable for 7SK stability *in vivo*, challenging the long-held belief that methylation is essential to 7SK stability. We identified a previously undiscovered, metazoan-specific motif (MSM) in Bin3 that might control transcription regulation independent of 7SK or P-TEFb in specific tissues. Additionally, we report for the first time that Bin3, Bin3^{Y795A}, and Bin3$^{\Delta MSM}$ all bind to the U6 snRNA *in vivo*, consistent with binding of Bmc1 to U6 in fission yeast, and MePCE to U6 in human tissue culture cells. In all, have elucidated a conserved role for Bin3/MePCE in metazoan physiology, found that methylation is dispensable for 7SK stability *in vivo*, and that a metazoan-specific motif in Bin3 may have a novel function in transcription regulation independent of the 7SK snRNP.

484F **Stumple activates Wnt signaling through BRG1 regulation** Kai Yuan[1], Nawat Bunnag[1], Sara n kassel[2], Ethan Lee[2], Yashi Ahmed[31]Molecular and Systems Biology, Dartmouth College, [2]Vanderbilt University, [3]Dartmouth College

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, we identify an E3 ubiquitin ligase, Stumple, as a novel positive regulator of Wnt/β-catenin signaling. RNAi-mediated depletion and conditional CRISPR-mediated mutagenesis of *Stumple* decreased Wnt/β-catenin signaling in multiple tissues. Upon Wnt stimulation, Stumple interacted with BRG1, a subunit of a chromatin remodeling complex, and induced BRG1 ubiquitination without changing its stability. Depletion of BRG1 also decreased Wnt/β-catenin signaling. How BRG1 ubiquitination activates the Wnt/β-catenin pathway is currently under investigation.

485F **Dynamic regulation of the mutualistic retrotransposon R2 maintains ribosomal DNA copy number stability** Jonathan O Nelson, Alyssa Slicko, Yukiko M YamashitaWhitehead Institute for Biomedical Research

While the majority of transposable elements (TEs) are considered genomic parasites, there is a small but growing subset of TEs identified to contribute to host functions. Uncovering the regulatory mechanisms that maximizes the positive effect of these 'mutualistic' TEs while mitigating their potential danger to the host genome is critical to understanding how such host-TE mutualism emerges. We recently found that the ribosomal DNA (rDNA)-specific retrotransposon R2 is a new mutualistic TE. rDNA repeats are susceptible to copy number (CN) reduction via intra-chromatid recombination, which could lead to ribosomal RNA insufficiency, but rDNA CN expansion that occurs in the male germline offsets such rDNA loses, maintaining rDNA throughout the species. We recently found that this rDNA expansion relies on the R2 retrotransposase, which is normally repressed, but becomes expressed when rDNA CN is reduced in order to induce rDNA expansion. We sought to identify the mechanisms of this dynamic rDNA-dependent R2 regulation to reveal the regulatory interactions that enable host-TE mutualism.

In order to uncover rDNA CN-dependent R2 regulators, we used single cell sequencing to identify genes that are differentially expressed upon rDNA reduction within germline stem cells — the major site of rDNA expansion. This analysis identified 211 up- and 147 downregulated genes when rDNA CN is low, which we consider candidate positive or negative R2 regulators, respectively. We are currently screening if RNAi knockdown of these candidate positive or negative regulators respectively inhibits or induces the reversion of the 'bobbed' dorsal cuticle defects stereotypical of rDNA insufficiency, a readout for rDNA CN expansion. One of the strongest hits from this preliminary screening in the insulin receptor (InR), which we found to be downregulated upon rDNA CN reduction. InR inhibition via either RNAi or expression of a dominant negative isoform dramatically induced R2 expression and rDNA CN expansion. These results suggest that this major metabolic pathway is modulated in response to changes in rDNA CN to modify TE regulation and maintain rDNA. We are now investigating the downstream insulin signaling factors to identify the specific mechanisms that control R2 expression. We propose that integration of mutualistic TEs into the host's signaling network is critical for ad hoc expression that allows for their beneficial activities.

486F **Mimicking Permanent Phosphorylation of HP1a Causes Sterility** James C Walts, Nicole C RiddleBiology, University of Alabama at Birmingham

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 modification, 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 and accumulates to similar levels as wildtype HP1a. Polytene chromosome analysis indicated that the phospho-mimic HP1a protein continues to localize to heterochromatic regions of the genome, including the centromeres and telomeres. 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, but no disturbance of HP1a localization on polytene chromosomes. These results suggest that phosphorylation of HP1a proteins at the site we modified (S88/89/91) might have specific functions in the Drosophila germline.

487F **Analysis of transcription factor binding sites upstream of the p38Kb gene in oxidative stress resistance**
Brooke Allen[1], Alysia Vrailas-Mortimer[21]Illinois State University, [2]Oregon State University

Under selection from their environments, organisms have evolved numerous mechanisms to resist environmental stressors (i.e., oxidative stress, pH change). We are currently living through the 6th mass extinction event to hit our planet, which is caused by human induced changes to the environment. Given the severity of the impending effects of climate change, it is imperative that we gain a better understanding of stress response mechanisms and how they work. Previous studies have investigated how stress resistant mechanisms evolve, but little is known about how selective pressures alter the regulation of these mechanisms. One environmental insult that organisms face is oxidative stress, which occurs when reactive oxygen species (ROS) production overwhelms antioxidant defenses. A highly conserved pathway to counteract oxidative stress is the p38 Mitogen Activated Protein Kinase (p38 MAPK or p38K) pathway. While the role of p38K post-translational modifications in response to oxidative stress is well studied, little is known about oxidative stress induced p38K transcriptional regulation. We have found that there are conserved binding sites for the transcription factors Activator Protein-1 (AP-1) and longitudinals lacking (lola-PT) in the promotor region of p38Kb. Both AP-1 and lola-PT mediate p38Kb transcription. lola-PT may be playing a role in oxidative stress response by promoting p38Kb transcription under stressful conditions by utilizing an induction mechanism. Whereas AP-1 plays a role in oxidative stress response by utilizing a buffering mechanism to promote excess p38Kb transcription under standard conditions that provides immediate protection for future oxidative stress exposure. We have generated transgenic lines in which we have scrambled the AP-1 site, the lola-PT site or both. We have found that the loss of sites impacts stress response and viability. The AP-1 site is important for providing immediate stress response but requires the presence of a lola-PT site to be viable, suggesting lola-PT acts as a repressor to regulate AP-1 expression under standard conditions. The lola-PT site is important for providing a strong stress response by using the induction mechanism. Overall, this data suggests that the timing of having excess p38Kb is important for both viability and stress response and that having too much p38Kb before the onset of stress is detrimental, whereas the induction of p38Kb in response to stress is beneficial.

488F **A screen for novel roles of G protein-coupled receptors in eye development** Romaisa Shahid[1], Mhamed Bashir[1], Brooke Cayting[1], Joseph Bunker[1], Matthias Schlichting[2], Jens Rister[11]University of Massachusetts Boston, [2]Northeastern University

Abnormal growth, a hallmark of cancer, is caused by a disruption of signaling pathways that control cell proliferation and apoptosis. In both humans and *Drosophila,* G protein-coupled receptors (GPCRs) are part of various signaling pathways that regulate cell proliferation and development. Moreover, dysregulation of these receptors has been shown to act as a driver for various human cancer forms. However, the role of GPCRs in normal eye development is not fully understood. Using the *Drosophila melanogaster* eye as a model, we aim to identify GPCRs that play a role in both early eye development (a growth context) and late eye development (a post-mitotic context). In late eye development, two color-sensing photoreceptor subtypes and their Rhodopsins (Rh5 and Rh6) are specified by the Hippo signaling pathway, whose dysregulation is also implicated in various human cancer forms.

Here, we performed a high-throughput CRISPR-Cas9 screen with gRNA lines for all known 122 *Drosophila* GPCRs. With the eye-specific driver *GMR*-GAL4, we screened for GPCRs whose knockout caused disruption to normal eye development: eye overgrowth, a rough eye, or a small eye. Moreover, we also screened for GPCRs whose knockout caused improper specification of the color-sensing photoreceptors. This would indicate a role for the GPCRs in regulating the Hippo pathway. We identified 11 GPCRs whose knockout caused a rough eye, while the knockout of 63 GPCRs caused mild roughness around the edges of the eyes. Additionally, we found a GPCR, *TkR86C*, whose knockout caused a dramatic increase in Rh5 photoreceptors. The expansion of Rh5 expressing photoreceptors was accompanied by co-expression of Rh6, indicating that *TkR86C* is required to repress Rh5 in the Rh6 photoreceptors. Since, Hippo pathway activity is required to repress Rh5 fate, we hypothesize that this GPCR acts as an upstream activator of the Hippo pathway. We will further analyze these candidates in both the *Drosophila* eye disc and wing imaginal disc to determine whether these GPCRs are required for eye development or whether they play a general role in regulating tissue growth and the Hippo

pathway. Taken together, our screen has the potential to unravel cancer relevant GPCRs that control growth and prevent tumors. Since GPCRs are ideal drug targets, this screen could inspire the development of novel drugs as well as therapies for cancer treatment.

489S **Evaluating SCRMshaw enhancer prediction for non-traditional model organisms through a cross-species reporter assay** Ellen Tieke[1], Hasiba Asma[2], Marc S. Halfon[3], Yoshinori Tomoyasu[11]Biology, Miami University, [2]Program in Genetics, Genomics, & Bioinformatics, University at Buffalo, [3]Departments of Biochemistry, Biological Sciences, and Biomedical Informatics, University at Buffalo

Enhancers are central to the spatial and temporal regulation of gene expression; however, enhancer discovery and validation are still challenging outside of well-studied model organisms, such as *D. melanogaster*. There is a growing number of insect species with fully-sequenced genomes, but little to no known enhancers within those species. The fully-sequenced genomes allow for two main methods for enhancer discovery: chromatin profiling techniques (e.g. ATAC-seq) and *in silico* enhancer prediction. Although a good starting point, open chromatin profiling requires additional analyses to identify enhancers since not every section of open DNA will be an enhancer sequence. *in silico* prediction can be a good alternative since it allows for rapid enhancer screening based solely on a genome sequence. However, TF-binding site-based analyses rely on conserved motifs, which can lead to many false positives for enhancer prediction. SCRMshaw (Supervised *cis*-Regulatory Module discovery), a motif-blind enhancer discovery technique, has been developed with the hypothesis that enhancers have a deep conservation of fundamental regulatory mechanisms not noticeable to the human eye or traditional alignment algorithms, but can be recognized through machine learning. By using previously identified *D. melanogaster* enhancer sequences as tissue-specific training sets, SCRMshaw predicts potential enhancer sequences within genomes across the holometabolous insect spectrum. This project evaluates the accuracy of SCRMshaw by looking at (i) novel enhancer predictions and (ii) predictions orthologous to previously identified *D. melanogaster* enhancers across three insect orders (Diptera, Coleoptera, and Hymenoptera). We chose 6 genes important for wings, one of the best studied contexts, and are testing the activity of 19 predicted enhancers in a cross-species setting. Our preliminary results suggest over 70% success in predicting enhancers with tissue-specific activity. These outcomes offer SCRMshaw as a promising enhancer prediction tool for annotating the expanding number of fully-sequenced genomes.

490S **Wing enhancers of *vestigial* evolved through modifications of the body wall enhancers** Jabale Rahmat, Yoshinori TomoyasuBiology, Miami University

The acquisition of wings is often regarded as the main driving force for the evolutionary success of insects. Although the exact tissues that contributed to the evolution of wings are still hotly debated, the wing is generally considered to have evolved from a part of the body wall and/or associated branch structures. But the detailed molecular mechanisms that facilitated the emergence of wings are still quite elusive. Decades of studies in *Drosophila* have revealed a large network of genes (wing gene network, WGN) that governs the development of wings. The *vestigial* gene (*vg*) is a key gene in the WGN, as misexpression of *vg* is sufficient to initiate wing development in some non-wing tissues in *Drosophila*. Interestingly, a network of several wing genes, including *vg*, also operates in the body wall of various insects and even non-insect arthropods, suggesting that a network similar to the WGN (preWGN) in the body wall precedes the emergence of insect wings. In the WGN, *vg* expression is regulated by several signal transduction pathways, such as Decapentaplegic and Wingless signals, through these signals act on the signal transduction response elements (STREs) within the wing enhancers of *vg*. Here we provide evidence that wing enhancers of *vg* evolved through modification of its body wall enhancers. We demonstrate that (i) wing and body wall enhancers of *vg* share the location at the *vestigial* locus, (ii) wing and body wall activities of these enhancers cannot be separated from each other without compromising their activities, (iii) STREs are essential for the wing activity of these enhancers but dispensable from their body wall activity. These results suggest that *vg* wing enhancers evolved by acquiring STREs within the body wall enhancers. We propose that the acquisition of STREs within the preexisting enhancers provides new connections within a gene regulatory network, facilitating the evolution of a new structure.

491S **Brat binds to mRNAs and accelerates their decay via a 3' decay pathway to control development** Robert Connacher[1], Yichao Hu[2,3], Richard Roden[1], Xiaohang Yang[3], Howard Lipshitz[2], Michael O'Connor[4], Aaron Goldstrohm[11]Biochemistry, Molecular Biology, & Biophysics, University of Minnesota, [2]Molecular Genetics, University of Toronto, [3]Institute of Genetics, Zhejiang University, [4]Genetics, Cell Biology and Development, University of Minnesota

TRIM-NHL proteins share a conserved domain architecture and play crucial roles in stem cell biology, fertility, and

development. The *Drosophila* TRIM-NHL protein, Brain tumor (Brat), plays a crucial role in the maternal-to-zygotic transition in embryos and neural stem cell differentiation in larval brains. Brat recognizes specific, single-stranded RNA motifs through its C-terminal NHL domain to destabilize and inhibit translation of bound mRNAs. In light of these observations, we sought to determine the biological relevance of RNA-binding and mechanism(s) by which Brat represses mRNAs.

Using CRISPR/Cas9 genome engineering, we mutated key RNA-binding residues in the *brat* coding sequence. RNA-binding defective mutants phenocopy the lethality observed in loss-of-function *brat* mutations. In the larval brain, Brat was previously reported to bind to and repress the mRNAs of key transcription factors during the asymmetric cell division of neural stem cells. Strong loss-of-function *brat* alleles are defective in neural stem cell differentiation and develop brain tumors as a result. Consistent with this model, mutants defective in RNA-binding also caused an over-proliferation of neural stem cells in the larval brain at the expense of differentiated progeny. These results demonstrate that the essential function of Brat derives from its ability to bind and regulate mRNAs.

To discover how Brat regulates mRNAs, we used cell-based reporter assays to analyze cis-acting RNA elements and identify transacting factors necessary for repression. Our data demonstrate that Brat represses mRNAs that contain Brat motifs in the 3'UTR; the extent of repression depends on the number and spacing of these motifs. Efficient repression also requires the 3' poly-adenosine tail of target mRNAs and enzymes involved in the 3' mRNA decay pathway, including the CCR4-NOT deadenylase complex. Finally, we mapped this repressive activity to previously uncharacterized domains of Brat.

Together, our data support a model in which Brat binds to mRNA 3'UTRs via its NHL domain, then recruits decay factors through several other domains. These mechanistic observations may be shared by other members of the TRIM-NHL family.

492S **Spatiotemporal regulation of *orthodenticle* during *Drosophila* embryogenesis and retinal development**
Sosina Abuhay, Grace Carey, Corey Jones, Marjorie Wang, Rhea DattaHamilton 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.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 otd locus - 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.

493S **Investigating the Role of RNA Editing in Toxin Exposure in D. *sechellia*** Neil Bohan[1], Jake Multer[2], Joseph Coolon[21]Biology, Wesleyan University, [2]Wesleyan University

Toxin exposure changes the transcriptome of *Drosophila* through attempts by flies' cells to mitigate damage or as a product of the toxin's action. Heat shock has been shown to cause regulated protein-catalyzed A-to-I (ADAR-catalyzed) and C-to-U (cytidine deaminase family-catalyzed) mRNA nucleotide edits in *Drosophila*. These edits can occur in all segments of mRNA, with the potential to alter protein product regulation and function. *Drosophilasechellia*, a species of fruit fly endemic to the Seychelles Islands, have specialized to primarily feed and oviposit on noni fruit, which possesses high levels of potent insect toxins such as octanoic and hexanoic acids as well as other plant defense compounds. While *D.sechellia* are able to process and resist high levels of toxins, other closely related species including sister species *D. simulans* are highly susceptible to them. The mechanism of this increased resistance in *D. sechellia* is poorly understood. By applying a custom-built bioinformatic pipeline to *D. sechellia* full-transcriptome RNA-Seq data, we are investigating whether the frequency of regulated A-to-I and C-to-U editing events changes in response to toxin treatment.

494S **Yorkie dependent transcriptional network promotes tumor growth.** arushi rai[1], Indrayani Waghmare[2], Amit Singh[1,3,4,5,6], Madhuri Kango-Singh[1,3,4,51]Department of Biology, University of Dayton, [2]Cell and Developmental

Biology, Vanderbilt University, [3]Premedical Program, University of Dayton, [4]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [5]Integrative Science and Engineering (ISE), University of Dayton, [6]Center for Genomic Advocacy (TCGA), Indiana State University

The Hippo pathway effector, Yorkie (Yki) is a key mediator of signaling interactions and transcriptional addictions in tumor cells and presents an attractive opportunity to study transcriptional dependencies in cancer cells. The Ras^{V12} $scrib^{-/-}$ tumor mosaic model, a well-established model, shows activation of oncogenic Ras in the background of impaired apical-basal polarity. Previously, we have shown that in Ras^{V12}, $scrib^{-/-}$ cells Wingless (Wg) acts upstream of Caspases, JNK and Yki, and via its canonical and non-canonical pathways interacts with Yki to promote tumor growth. Our goal is to further understand how the two evolutionarily conserved signaling pathways i.e., Hippo and Wingless crosstalk and interact with each other using the Ras^{V12}, $scrib^{-/-}$ tumor model in *Drosophila* imaginal discs. Our data showed that the *wg* transcriptional reporters and *wg* transcript levels both are upregulated in Ras^{V12}, $scrib^{-/-}$ cells. In other contexts, *wg* is shown as a transcriptional target of Yki. Therefore, we will test for (a) the effects of modulating Yorkie, the main effector of the Hippo pathway, on the transcription and expression of Wg and other Wg pathway components by reporter assays and qRT-PCR-based approaches, and (b) the effect of modulating Wg pathway components (Frizzled, TCF) on intrinsic Wingless signaling and also growth of Ras^{V12}, $scrib^{-/-}$tumors, and (c) feedback interactions that promote tumorigenesis using genetic epistasis-, and immunohistochemistry-based approaches. Here, we present our progress on the organization of the molecular network involving Wingless and Yorkie.

495S **Interactions among *cis*-regulatory regions of cell fate determinants during follicle cell patterning** Kelvin Ip[1], Scott De Vito[1], Baptiste Rafanel[2,3], Kaitlin Mac Donald[1], Mariana Fregoso Lomas[4], Laura Nilson[11]McGill University, [2]Institute of Molecular Biotechnology of the Austrian Academy of Sciences, [3]Vienna BioCenter PhD Program, [4]Novartis Canada

Patterning of the Drosophila follicular epithelium requires the concerted action of multiple localized extracellular ligands. In early development, the EGFR ligand Gurken (Grk) is found at the oocyte posterior where, in the presence of Unpaired (Upd), it induces the posterior determinant *midline* (*mid*) in the overlying follicle cells. Later, Grk is found in the anterior where, in the presence of Dpp, it instead induces the dorsal anterior determinant *mirror* (*mirr*). The choice between these targets is reinforced by double negative feedback between *mid* and *mirr,* and by repression of *mid* by Dpp, and *mirr* by Upd. This system highlights the importance of cellular context in determining signaling outcome, but how these inputs are integrated at the level of the *mid* and *mirr cis*-regulatory elements remains unclear.

We identified genomic regions from the *mid* and *mirr* loci that drive expression of a transgenic reporter with a pattern resembling that of the endogenous genes and therefore may reveal important *cis*-regulatory elements. For *mid*, we found two non-overlapping regions (“mid-up” and “mid-in”) that each drive posterior follicle cell expression similar to that of endogenous *mid*. Despite their similar expression, neither reporter captures all the inputs that regulate endogenous *mid*: mid-up is induced by Upd and repressed by Mirr while mid-in is repressed by Dpp.

Mutation of putative binding sites for the Dpp signaling effector MAD in the mid-in reporter (mid-inΔMAD) results in reporter expression in all columnar follicle cells, suggesting that these sites are required for Dpp to repress anterior mid-in expression and therefore define a silencer element. Interestingly, mid-inΔMAD is activated, rather than repressed, by Dpp signaling suggesting that mid-in also contains a positive Dpp response element that is normally overridden by the putative silencer element. In support of this idea, when we divide mid-in in half, the two halves (mid-in.1 and mid-in.2) behave differently: mid-in.2, which contains the putative silencer element, is restricted to the posterior and repressed by Dpp, like full-length mid-in, whereas mid-in.1 is expressed in all columnar follicle cells and is activated by Dpp, like mid-inΔMAD.

Together these data suggest that: a) in the context of mid-in *cis*-regulatory region, both the anterior expression of mid-in.1 and its activation by Dpp are suppressed by the presence of mid-in.2 sequences and b), that this “enhancer dominance” is mediated by the putative silencer element in the mid-in.2 region.

496S **A role for Set1 at Zygotic Genome Activation** Natalie Gilbert Gonzaga-Saavedra, Shelby BlytheNorthwestern University

Prior to zygotically controlled transcription, embryos rely on maternally supplied RNAs and proteins to drive the cell cycle, patterning, and transcription. During Zygotic Genome Activation (ZGA) bulk zygotic transcription occurs, and in Drosophila embryos, there is de novo binding of Pol II to over 4,000 promoters. Before ZGA, DNA replication was rapid

(<10min) and maternally driven. During ZGA (Nuclear cycle 13), replication time is lengthened to 19min and includes a DNA replication checkpoint. In DNA damage response (ATR) mutants, the cell cycle time remains short but results in lethality during NC14 due to incomplete replication in NC13. Interestingly, interfering with ZGA regulators Zelda and GAGA suppresses ATR lethality. We find that Set1, an H3K4 methyltransferase, also suppresses ATR lethality, leading to the hypothesis that Set1 may be a regulator of ZGA. To characterize the role of Set1 in ZGA we have live-imaged a sfGFP tagged Set1 and find Set1 nuclear localization begins at NC12. Staged embryo ChIP-seq reveals that H3K4me2 and me3 occupy genes beginning at NC13. While Set1 occupancy correlates with initiating RNA PolII and H3K4me2/3, a subset of the earliest expressed genes lack this methylation despite being bound by Set1. Through identification of Set1's methyltransferase dependent and independent roles we will contribute to the understanding of both cell cycle and gene regulation at the onset of zygotic transcription. This research is funded by NIH/NICHD, Pew Charitable Trusts, and Northwestern University.

497S **Fat cadherin cleavage releases a transcriptionally active nuclear fragment to regulate Yki targets** Jannette Rusch[1], Chikin Kuok[2], Nattapon Thanintorn[1], Yonit Tsatskis[2], Helen McNeill[11]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. We have found that FtICD is present in the nucleus in tissue culture cells, 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 the anti-apoptotic gene, *Diap1*, a target of the Hippo pathway. In vivo analysis of the *Diap1* enhancer, HRE (Hippo Response Element), indicates that FtICD can activate the Diap1 enhancer element, supporting a nuclear function for *ft*. We propose that the nuclear function of Ft functions to modulate Hippo pathway activity, complementing its established function as an upstream regulator of Hippo signaling.

498S ***miR-277* ameliorates Aβ42-mediated neurodegeneration in *Drosophila* eye model of Alzheimer's Disease** Prajakta Deshpande[1], Chao-Yi Chen[2], Anuradha V Chimata[1], Catherine Yeates[1], Chun-Hong Chen[2,3], Madhuri Kango-Singh[1,4,5,6], Amit Singh[1,4,5,6,71]Department of Biology, University of Dayton, [2]Institution of Molecular and Cellular Biology, National Taiwan University, [3]National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, [4]Premedical Program, University of Dayton, [5]Center for Tissue Regeneration & Engineering (TREND), University of Dayton, [6]Integrative Science and Engineering (ISE), University of Dayton, [7]Center for Genomic Advocacy (TCGA), Indiana State University

Alzheimer's disease (AD), an age-related progressive neurodegenerative disorder, exhibits reduced cognitive functions with no cure to date. One of the reasons for AD is the extracellular accumulation of Amyloid-beta 42 (Aβ42) plaques. We misexpressed human Aβ42 in the developing retina of *Drosophila*, which exhibits AD-like neuropathology. Accumulation of Aβ42 plaque(s) triggers aberrant signaling resulting in neuronal cell death by unknown mechanism(s). We screened for microRNAs (miRNAs) which post-transcriptionally regulate expression of genes by degrading mRNA of the target genes. In a forward genetic screen with candidate miRNAs, we identified *miR-277* as a genetic modifier of Aβ42-mediated neurodegeneration. Gain-of-function of *miR-277* rescues Aβ42-mediated neurodegeneration whereas loss-of-function of *miR-277* enhances Aβ42-mediated neurodegeneration. Moreover, misexpression of higher levels of *miR-277* in the GMR>Aβ42 background restores the retinal axonal targeting indicating functional rescue. Here, we provide a mechanism of how *miR-277* modulates Aβ42-mediated neurodegeneration and demonstrate its neuroprotective role in Aβ42-mediated neuropathology.

499S **Structure-function analysis of Defective proventriculus (Dve) in *Drosophila melanogaster* eye growth and development** Anuradha V Chimata[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,51]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

Axial patterning is required to delineate Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes in a sequential manner to transform a monolayer organ primordium to a three-dimensional organ. During eye development,

DV axis is the first lineage restriction event and any deviation results in developmental birth defects. During this process, generation and establishment of morphogen gradient plays important roles such as negative regulator of eye development, growth and determination of the eye versus head fate. Previously, we have identified *defective proventriculus (dve,* an ortholog of SATB1*)* as a novel dorsal patterning gene that regulates *wingless (wg)* transcription. Loss-of-function of *dve* results in dorsal eye enlargement and decrease in Wg, while gain-of-function of *dve* results in eye suppression and increased *wg*. In addition to eye versus head fate, SATB1, the human ortholog of *dve* is highly upregulated in cancers and is known to activate various signaling pathways to trigger growth. Here we present our structure-function analysis to determine the domains of Dve required for growth regulation and Wg mediated determination of eye versus head fate. Dve has a ULD domain for tetramerization, two HOX domains for DNA binding and a PPP4R2 domain for H2AFX dephosphorylation. We have developed several transgenic fly lines, which will allow us to ectopically induce expression of the specific Dve protein domain(s) alone or in combination and assay their effect in the eye. Our preliminary data suggests that HOX domain alone can induce *wg* expression and exhibit weak eye suppression phenotype, but it is not enough to fully exhibit Dve mediated eye suppression function during development. We will present data from our studies to dissect the growth versus eye fate function of Dve protein domain(s) using this structure-function analysis. Understanding this can increase our knowledge of which domains might be dispensable or pathogenic in human development or disease.

500S **Gene Regulatory Networks in Development: Genetic Variation and Robustness of Anteroposterior (AP) Axis Formation in *Drosophila*** Lossie (Elle) Rooney[1], Prasad Bandodkar[2], Cranos Williams[3], Gregory T. Reeves[21]Biological Sciences (Genetics), NC State University, [2]Chemical Engineering, Texas A&M University, [3]Electrical & Computer Engineering, NC State University

Body plan patterning is a critical step in embryonic development that has health and viability consequences across the lifespan. Anteroposterior (AP) axis formation is an early event in body plan patterning that establishes the head-to-tail orientation for determining cell and tissue fates. In Drosophila melanogaster, Bicoid is a well-studied transcription factor that acts as a morphogen in AP axis patterning by influencing expression of the Gap genes in a concentration-dependent manner to create distinct expression profiles. The Gap genes influence additional target genes that also show distinct expression profiles. Though this system has been studied extensively and many of the relevant genes have been identified, the mechanisms that allow robustness of AP axis formation across genetic backgrounds are not well-characterized. We address this gap using the natural variation of the *Drosophila melanogaster* Genetic Reference Panel (DGRP). By quantifying spatial expression patterns of AP genes across lines of the DGRP, we can identify genetic backgrounds that show significant changes in expression that may be due to altered regulation of AP genes. We present imaging data for ~70 lines of the DGRP and QTLs under investigation.

501S **Epigenetic effects of transgenerationally inherited piRNAs** Peiwei Chen, Alexei AravinCalifornia Institute of Technology

In *Drosophila*, piRNAs inherited transgenerationally from the mother can endow the offspring with unique silencing capacities of cognate targets. Such a piRNA-mediated epigenetic effect was demonstrated by reciprocal crosses between flies that either have or lack the active copies of a transposon throughout the genome (where the specific locus responsible for the epigenetic effects of piRNAs remains unmapped) and by reciprocal crosses between flies that either have or lack a transgenic piRNA-encoding locus. To date, epigenetic effects of transgenerationally inherited piRNAs produced from a natural, defined locus have not been demonstrated. Furthermore, it remains unknown whether transgenerationally inherited piRNAs act differently in the two sexes, as prior work only examined females. Here, using spontaneous sex chromosome nondisjunction to generate XX and XXY sisters, we modified the maternally supplied piRNA pool without transgenics, where the differences could be precisely attributed to the Y chromosome – a natural and defined region in the genome. Supplying Y-derived piRNAs through the oocyte profoundly changed the global piRNA ping-pong and phasing patterns in the offspring, indicating differential processing of piRNA precursors in the cytoplasm. Despite receiving the same piRNAs from the mother, male and female progeny exhibited distinct changes in their piRNA processing landscape, suggesting that the epigenetic effects of transgenerationally inherited piRNAs depend on the recipient's genetic makeup and sexual identity. We also systematically profiled the chromatin landscape (RNA pol II, H3K4me2, H3K9me3, and HP1d/Rhino) of Y-linked piRNA loci in the presence of maternal Y piRNAs, pinpointing the nuclear functions of transgenerationally inherited piRNAs. Taken together, using a natural and defined genomic region to modify maternally supplied piRNAs, we elucidated the sex-specific, multifaceted epigenetic actions of transgenerationally inherited piRNAs.

502S **Activin signaling network in Drosophila tissue growth and development** Yisi Louise Lu, Hiroshi Nakato, Michael O'ConnorDepartment of Genetics, Cell Biology and Development, University of Minnesota

In the Drosophila TGF-β signaling pathway, three Activin-specific ligands, Myoglianin (Myo), Activin-β (Actβ), and Dawdle (Daw), have been implicated in the regulation of different physiological activities in a tissue specific manner. For example, loss of actβ results in small muscles with altered carbohydrate metabolism and changes in NMJ electrophysiology. Loss of daw results in major metabolic disfunction including altered carbohydrate metabolism and a disruption in the TCA cycle, while loss of myo leads to smaller brain and imaginal disc sizes.

Although loss of each Activin ligand gives rise to a unique phenotype, all three ligands signal through dSmad2, a common intracellular transcriptional transducer. Our goal is to elucidate the mechanisms by which the three Activin ligands produce specific responses and phenotypic outcomes in different tissues. We have examined the cross-regulation among the three Activin ligands by RT-qPCR studies and by employing transgenic GFP reporter fly lines. Our results suggest that Daw is negatively auto-regulated by dSmad2 while Myo may be positively autoregulated and that these ligands likely cross-regulate each other's expression in a tissue-specific manner, such as loss of actβ upregulating Myo in the larval brain and muscle. We have also performed transcriptomic analyses in different fly tissues by using a temporal expression system that allows us to specifically turn on activated Babo type I receptor. By these and other studies, we have found that Myoglianin and Ecdysone signaling act together to facilitate optic lobe development. These studies will provide a deeper understanding of the TGF-β Activin signaling network in controlling important developmental activities such as metamorphosis and physiological homeostasis.

503S **Using CRISPR/Cas9 genome editing to dissect the 5' regulatory region of the gene *hindsight*** Richard Do, Bruce H. ReedBiology, University of Waterloo

The gene *hindsight* (*hnt*) encodes a transcription factor and is expressed in multiple tissues during *Drosophila* embryogenesis. In certain contexts, *hnt* has been identified as a Notch responsive target gene and is upregulated by the transcription factor Suppressor of Hairless (Su(H)). In the extraembryonic tissue known as the amnioserosa, *hnt* expression is required for tissue maintenance and successful germ band retraction; *hnt* expression is downregulated in this tissue concurrent with programmed degeneration. It remains largely unknown how this dynamic expression of *hnt* is regulated. We are using CRISPR/Cas9 genome editing to dissect the large 5' regulatory region in order to define cis elements that regulate *hnt* expression in the amnioserosa and other embryonic tissues. CRISPR/Cas9 provides an effective tool to create multiple transgenic mutants that carry precise deletions in the regulatory region of *hnt*. Each transgenic mutant is designed to create an ~5 kb deletion targeted to putative Su(H) transcription factor binding sites. Following this approach, immunostaining and phenotype analysis of mutant embryos will confirm any changes in *hnt* embryonic expression eventually leading to a better understanding of the cis-regulatory region of this gene.

504S **Combined inputs of two antagonistic transcription factors regulates progenitor vs. photoreceptor cell fate decision in the developing Drosophila eye** Suzy Hur[1], Andrea Herman[2], Hernan G. Garcia[2,3,4,5], Ilaria Rebay[11]Ben May Department for Cancer Research, The University of Chicago, [2]Department of Physics, University of California at Berkeley, [3]Department of Molecular and Cell Biology, University of California at Berkeley, [4]Institute for Quantitative Biosciences-QB3, University of California at Berkeley, [5]Chan Zuckerberg Biohub

The regulation of genes in multicellular organisms is generally achieved *through the combinatorial activity of different transcription factors*. How the input concentrations of multiple transcription factors are interpreted at developmental gene enhancers to regulate precise cell fate decisions is unclear. Pointed (Pnt) and Yan are a pair of antagonistic transcription factors that regulate numerous cell fate decisions downstream of RTK/MAPK signaling pathway in Drosophila. Pnt and Yan compete for the same DNA binding site and generally exert activating and repressive inputs on their target genes respectively. Previously, we have shown that the ratio of Pnt-to-Yan protein levels in the nucleus, not the absolute level of either factor, determines whether cells remain in the progenitor state or differentiate into photoreceptor cell fates in the developing retina. However, how the concentration ratio of the two factors regulates mRNA expression levels of target genes during the progenitor vs. photoreceptor cell fate decision is unknown. To address this, we simultaneously measured Pnt and Yan protein levels as well as the mRNA level of the R7 photoreceptor gene *prospero*(*pros*), a known target of Pnt and Yan, in hundreds of single cells making the Progenitor vs. R7 cell fate decision. Our results show that initiation of *pros* transcription only occurs in cells with sufficiently high Pnt-to-Yan ratio. We are currently combining experimental data with mathematical modeling to investigate which transcriptional bursting parameter may be under the regulation of Pnt-to-Yan ratio, and how the ratiometric control strategy is utilized by

developing cells to make accurate and reliable fate decisions.

505S **Transcriptome analysis of the effects of loss of *ft* and of deletion of conserved domains.** Nattapon Thanintorn[1], Jannette Rusch[1], Megan Glaeser[1], Yonit Tsatskis[2], Yi Qu[3], Hongtao Zhang[3], Hyunseo Oh[4], Helen McNeill[11]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 across several organisms. Loss of *ft* in flies leads to pupal lethality, massive overgrowth of imaginal discs, and loss of planar cell polarity (PCP) in tissue. 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 conserved regions of *ft* contribute Fat function in growth control, planar polarity, mitochondria function, and metabolism. We have used extensive CRISPR-based mutagenesis of Drosophila Ft to dissect Ft function *in vivo*. Both conserved domains and functional-domain regions were deleted. Here we present the effects of loss of different domains of Ft, and conduct transcriptome analysis of 8 CRISPR-based mutagenesis of Ft in imaginal wing disc to i) identify differentially expressed genes, ii) uncover potential functions of *ft*, and iii) analyze downstream effects in growth regulation and metabolism in *ft* mutants. Understanding how Fat regulates tissue growth and organization will generate new hypotheses to better understand how mutations of Fat cause human diseases.

506S **Synthetic reconstruction of the *hunchback* promoter specifies the roles of Bicoid, Zelda and Hunchback in the dynamics of its transcription** Gonçalo Fernandes[1], Huy Tran[1,2], Mathieu Coppey[3], Aleksandra M Walczak[4], Nathalie Dostatni[11]Nuclear Dynamics unit, Institut Curie, [2]École normale supérieure, [3]Laboratoire Physico Chimie, Institut Curie, [4]Laboratoire de Physique, École Normale Supérieure

In many developmental systems, cell identity is determined by morphogen gradients providing concentration-dependent positional information along polarity axes. Although the critical role of these gradients is well recognized, it is unclear how they can provide reproducible expression patterns despite the stochastic nature of transcription. To address this question, we studied the response downstream of the Bicoid (Bcd) morphogen gradient in *Drosophila* embryos, focusing on its main and earliest target gene, *hunchback* (*hb)*.

Using the MS2-MCP system to fluorescently tag nascent mRNA, transcription dynamics were analysed at high spatiotemporal resolution in living embryos. Adapting this approach to synthetic MS2 reporters with various combinations of DNA binding sites, we highlighted the roles of Bcd and its partners, Hb and Zelda (Zld), in the transcription mechanism. Expression of a reporter with only nine Bcd binding sites almost reproduces the hb-MS2 reporter's pattern, except for the very steep expression boundary and the speed to reach steady-state. This suggests that Bcd alone defines the positioning of the boundary, partially its steepness but not the speed of its establishment. In addition, binding of Bcd's partners to the promoter speed-up the process by acting in different steps of the transcription mechanism: i) Hb synergizes with Bcd by reducing transcription burstiness and increasing the polymerase firing rate; ii) Zld lowers the Bcd concentration threshold required for Bcd-dependent expression.

In collaboration with physicists, a biophysical model of Bcd-dependent expression was developed providing a theoretical framework for the experimental data. This model showed that the very rapid establishment of the *hb* expression boundary can be solely explained by an equilibrium involving the binding of Bcd molecules to their DNA-binding sites for positional information and requiring Zld and Hb for its temporal dynamics.

Finally, reducing the dose of Bcd by half and quantifying the corresponding shifts of Bcd-dependent reporter boundaries confirmed that Bcd is the main source of positional information for *hb* expression but argues that the decay length of the protein gradient is larger than the decay length of the transcriptionally active protein.

507S **Exploring how cells randomly choose between fates in the fly eye** Christina Im, Alison Ordway, Lukas Voortman, Robert JohnstonBiology, Johns Hopkins University

Development of an organism is complex and requires many different processes to occur simultaneously. Although many developmental processes are carefully regulated to yield stereotypical patterns, some occur stochastically. A stochastic process refers to one that involves a random variable and generates a random, unique outcome. 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, Rh4 is expressed whereas in Ss^{OFF} cells, Rh3 is expressed. *Ss* is expressed dynamically during development. This dynamic expression is controlled by multiple *cis*-regulatory elements, including two enhancers, 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. How these enhancers regulate *spineless* expression remains poorly understood. My research project focuses on dissecting the *late enhancer* in its endogenous location to determine how it regulates *ss* expression during stochastic cell fate specification.

508S **Regulation of the Mef2 transcription factor during myogenesis by the Class IIa Histone Deacetylase, HDAC4**
Sean A Hubbert, Michael V TaylorSchool of Biosciences, Cardiff University

The differentiation of genomically identical cells into diverse cell types observed in multicellular eukaryotes is underpinned by differential gene expression, controlled in large part by the activity of transcription factors (TFs). TFs are DNA-binding proteins that recognise and bind to specific cis-regulatory modules (CRMs), thereby regulating gene expression by either activating or repressing the transcription of their target genes. Our lab focuses on the role of one transcription factor, Myocyte enhancer factor 2 (Mef2), during muscle development. In *Drosophila*, *Mef2* is required for myogenesis during both embryogenesis to produce the larval musculature, and metamorphosis to produce adult muscles. Furthermore, its over-expression can also induce a premature differentiation phenotype in muscle progenitor cells. However, Mef2 does not function in a binary mechanism, in which its expression immediately induces differentiation of myoblast populations. Instead, *Mef2* is expressed long before many of its target genes, and transcription of target genes is also activated at different times throughout myogenesis. Together, this suggests that a mechanism of spatio-temporal regulation must control Mef2 transcriptional activity during myogenesis.

To address this issue, we are analysing the role of Class IIa Histone Deacetylases (HDACs) which, unlike the better-known Class I HDACs, do not possess intrinsic deacetylase activity. Previous *in vitro* work has identified Class IIa HDACs as negative regulators of Mef2 transcriptional activity during mammalian muscle cell differentiation. However, prior to this project, there has been no *in vivo* analysis investigating the role of Class IIa HDACs in Mef2 regulation during muscle development. *Drosophila* possess only a single *Mef2* and class IIa HDAC gene, *HDAC4*, compared to four mammalian *Mef2* and Class IIa HDAC genes, making it a favourable model for genetic analysis of these two proteins during myogenesis. We have found that over-expression of *HDAC4* can inhibit the formation of both the larval and adult musculature, and this is dependent upon an intact conserved Mef2-binding domain in HDAC4. We are currently using a CRISPR-Cas9 genome engineering approach to probe the role of endogenous HDAC4 in the regulation of Mef2 during myogenesis.

509S **Mechanisms controlling cell type-specific transcription factor activity** Ross Munce[1], Richard S Mann[21]Genetics and Development, Columbia University, [2]Columbia University

To ensure proper morphogenesis and cell fate specification, animals must generate highly stereotyped spatial and temporal patterns of gene expression. To this end, transcription factors (TFs) bind DNA regulatory elements such as enhancers to activate or repress transcription in particular cell types at particular developmental stages. Many TFs are expressed in several cell types at multiple stages throughout animal development. These TFs modulate different gene regulatory networks (GRNs) in different cell types, allowing a single TF to specify multiple cell fates. It is unclear how individual TFs are able to perform distinct functions in different cell types. One example of such a TF is the Drosophila Hox TF Ultrabithorax (Ubx). Ubx specifies third thoracic (T3) segmental identity by binding thousands of enhancers to regulate hundreds of genes, modifying the ground-state second thoracic segment (T2) GRN. T3 is highly modified at all positions along the proximal-distal (PD) axis relative to the serially homologous T2, including morphological changes to the body wall, hinge, and appendage proper. All of these changes must ultimately be due to Ubx activity. We have recently shown that Ubx transcriptional regulatory and chromatin remodeling activity is spatially segregated along the PD axis in T3. A complex of Ubx and its cofactors, Homothorax (Hth) and Extradenticle (Exd), functions as a transcriptional repressor in cells that express the zinc finger TF Teashirt (Tsh) and functions as a transcriptional activator in cells that are tsh-. Here, we provide evidence that Tsh mediates repressive activity by the Ubx-Hth-Exd complex at least partially by recruiting the co-repressor C-terminal Binding Protein (CtBP) to the complex. RNAi knockdown of CtBP diminishes Ubx repressive activity in the tsh domain of T3. Ectopic expression of wild-type tsh is sufficient to induce Ubx repressive

activity in a hth-dependent manner, while ectopic expression of a mutant form of tsh that cannot interact with CtBP (tshΔPLDLS) has reduced ability to induce Ubx repressive activity. Future work will assess the necessity of tsh and the PLDLS motif in Ubx-mediated transcriptional repression. These results support a model in which cell type-specific TFs can modulate GRNs by recruiting general transcriptional effectors to target genomic loci through interaction with highly conserved short peptide motifs.

510S **Poly(ADP-ribose) enzymes temporally regulate the expression of developmental genes.** Guillaume Bordet[1], Gbolahan Bamgbose[2], Iaroslava Karpova[2], Alexei Tulin[21]University of North Dakota, [2]Epigenetics department, University of North Dakota

Poly(ADP-ribosyl)ation, the synthesis of homopolymer ADP-ribose chains on the nuclear proteins, is pivotal for many nuclear functions, including chromatin remodeling that precedes transcription activation, repression, and epigenetic bookmarking. Two groups of enzymes are directly involved in this process: poly(ADP-ribose) polymerase (PARP) that assembles poly(ADP-ribose) (pADPr) polymers and poly(ADP-ribose) glycohydrolase (PARG) that degrades them into free ADP-ribose. While the roles of PARPs have been exhaustively studied, the specific function of glycohydrolase has yet to attract much attention. It is commonly assumed that PARG remains perpetually active throughout the cell cycle and cleaves pADPr at a constant rate. Consequently, changes in pADPr levels have only been attributed to up- and downregulation of PARP activity. We found that PARG activity is regulated by two distinct mechanisms: 1) PARG protein activity *in vivo* is regulated by the phosphorylation of two domains. 2) PARG subcellular and subnuclear localization and dynamics is regulated by the unstudied PARG C-terminal domain. Furthermore, we found that PARG binds without PARP to the gene body of silent genes to repress their expression and binds with PARP to the promoter region of active genes, suggesting that PARG plays two different roles in regulating gene expression.

511V **Coexpression analysis reveals candidate regulators of transposable element (TE) expression** Matthew Lawlor, Weihuan Cao, Christopher EllisonDepartment of Genetics, Rutgers University

The role of transcription factors and cofactors in the regulation of transposable elements (TEs) is not well understood in Drosophila. Some related TE families show similar expression patterns and tissue specificity, raising the possibility that host-encoded trans-acting factors beyond small RNA pathways contribute to regulation of TE expression. Under this hypothesis, TE expression would mirror natural variation in expression of such host-encoded factors. To test this hypothesis, we quantified host gene and TE expression in publicly available RNA-seq of 200 Drosophila Genetic Reference Panel strains. We identified coexpressed TEs and host genes and estimated the contribution of confounding factors such as TE copy number, retained intronic insertions, and pervasive transcription to variation in TE expression. Our analysis recovers known TE regulators, including components of the piRNA pathway, but we also find that transcription factors and cofactors are often coexpressed with TEs. We performed RNA-seq after tissue-specific RNAi of candidate regulators of TE expression identified by coexpression analysis. This approach validates two novel candidate TE regulating factors. We additionally report a broader list of coexpressed TEs and genes that will shed light on host/TE co-evolution, including mechanisms of cell type-restricted TE expression, and identify candidate TEs that may contribute to the expansion of host gene regulatory networks.

512V **Genetic Compensation between Ribosomal Protein paralogs mediated by a cognate circular sisRNA in *Drosophila*** Amanda Y. E. Ng[1,2], Jun Wei Pek[1,21]Temasek Life Sciences Laboratory, [2]Department of Biological Sciences, National University of Singapore

Regulation of gene expression is non-autonomous, especially with mounting evidence of inter-regulation between related genes, such as with paralogs like ribosomal proteins (RPs). This has been proposed to aid in paralog-specific effects by titrating the paralogs' expression levels. This idea is supported by paralog heterogeneity where RP paralogs have distinct expression and ribosomal incorporation patterns. However, the molecular mechanism of how paralogs communicate to modulate their expression levels remains unknown. Here, we use the *Drosophila melanogaster* RP paralogs RpL22 and RpL22-like for our study, specifically in the male germline where RpL22-like is the dominant RP incorporated into the ribosomes. We have found evidence of mutual repression between RpL22 and RpL22-like mediated by the circular stable intronic sequence RNA (sisRNA) sisR-9 which is produced from the RpL22 locus. SUMOylated RpL22 is known to be excluded from the ribosome, supporting an extra-ribosomal nuclear function for RpL22 which we found represses RpL22-like in trans, likely at the transcriptional level. RpL22 also represses itself in cis by interacting with its own pre-mRNA, independent of its SUMOylation. Knocking down SUMOylation disrupts the nuclear tri-lobal structure of RpL22, and the resulting upregulation of RpL22-like supports a repressive role for SUMO-RpL22 linked to its nuclear

localisation. This repression is found to be mediated by binding between RpL22 and sisR-9, which in turn is regulated by RpL22-like that can sequester sisR-9 in the cytoplasm, thereby indirectly repressing RpL22 function. This inter-paralog regulatory circuit enables genetic compensation for physiological adaptation to the loss of a RP which manifests as a change in their nuclear-cytoplasmic localisation that relates to RP function, either extra-ribosomal in the nucleus or ribosomal in the cytoplasm. For example, RpL22-like loss is mitigated by RpL22 decreasing its nuclear-to-cytoplasmic ratio to compensate for RpL22-like in the cytoplasm. We thereby propose a model of regulating RpL22 and RpL22-like expression via control of their nuclear and cytoplasmic localisation by their interaction with sisR-9, to ultimately ensure robust male germline development in *Drosophila.*

513V **Analysis of functionally enriched transcripts differentially associated with eRpL22-family polysomes** Caroline E Pritchard, Vassie C WareBiological Sciences, Lehigh University

The *Drosophila melanogaster* eRpL22 ribosomal protein (Rp) family contains two structurally divergent & developmentally essential paralogues: eRpL22 and eRpL22-like - the latter exhibits tissue-specific expression across development; the former is ubiquitously expressed. Multi-tissue co-localization comparison of eRpL22-like and core ribosomal components indicates eRpL22-like may have functional roles both within the ribosome itself and apart from ribosomal processes.

Sequencing of RNAs enriched on eRpL22 and eRpL22-like polysomes in adult testes revealed differential enrichment of mRNAs suggesting that paralogue-specific “specialized ribosomes” translate specific mRNAs. Functional enrichment analysis guided investigation into specific tissues by physiology (where to look) and function (what processes, pathways, and programs function differently in Rp mutants). KEGG pathway analysis revealed transcripts differentially enriched on eRpL22-like polysomes were uniquely implicated in pathways not canonically associated with ribosomal functions, including RAS/MAPK, WNT/PCP, and JAK-STAT signaling. Some pathways were over-represented in both polysome types, but these were derived from unique transcripts. Specific morphological defects within eRpL22-like knock-out ovaries correlate with functional enrichment analysis findings at the pathway, tissue-region, and sub-cellular structure levels.

Transcripts of genes functionally implicated in human disease, given by Human Phenotype Ontology (HPO) term association, were over-represented on eRpL22-like polysomes. Grouping each term into broad categories revealed HPO terms within the genitourinary, musculoskeletal, and nervous systems, and the brain, eye, head and jaw regions, were most functionally enriched on eRpL22-like polysomes. We have previously shown differential expression of eRpL22-like protein and specific knock-down phenotypes in several analogous regions in the fly.

Populations of transcripts differentially enriched on each polysome type share several unique transcription factor binding motifs. The mechanism linking these transcript populations to ribosomes containing an eRpL22-family Rp remains elusive.

Taken together, these data provide a foundation for uncovering mechanisms behind specialized ribosomes and/or specialized roles for eRpL22-family Rps.

514V **CRISPR Knock-in Split Fluorescent Protein as an Endogenous Tag in Drosophila melanogaster** Yingshan Bi, Wanpeng Wang, Tom KornbergCVRI, UCSF

Previous studies discovered that the beta-barrel between strands 10 and 11 of fluorescent proteins like mNeonGreen(mNG) can be split into two fragments and can also spontaneously self-complement into a functional fluorescent protein. In this case, the short split fragment mNG11 can serve as an endogenous tag integrated into a gene of interest. By knocking in the other 2(1-10) fragments, we can use this tool to detect cell-cell interactions, especially for proteins of low expression levels. To tag our proteins of interest endogenously, we first established a stably expressed mNG2(1-10) cell line. Next, we assembled CRISPR Cas9:sgRNA ribonucleoprotein and transfected the cells with mNG11 as a single-stranded oligonucleotide donor via nucleofection. This was modified from the protocol previously performed in mammalian cells and would be the first successful knocked-in the Drosophila cell line. We optimized the method so that the cell can recover faster and avoid potential contaminations during cell expansion. It will be helpful to move forward with flow cytometry cell sorting or other large-scale experiments. Furthermore, we performed a knock-in with a mutated and brighter version of mNG3A(1-10) and 3K(1-10) based on a previous study, and a consistent result was observed in our Drosophila cell line as it showed in mammalian cells. Increasing the fluorescent signals in the endogenous tag can help identify the molecular interactions among cell-cell signaling. The method hopefully can contribute to the exploration of the molecular and spatial network of proteomes by tagging the protein endogenously.

515V ***In vivo* contribution of the DPE core promoter motif to transcriptional regulation in developing *Drosophila melanogaster* embryos** Anna Sloutskin[1], Dekel Itzhak[1], Hadar Alter[1], Hadar Shachar[1], Manfred Frasch[2], Sascha H Duttke[3], Tamar Juven-Gershon[11]Bar Ilan University, [2]Friedrich-Alexander University of Erlangen-Nürnberg, [3]Washington State University

Regulation of transcription by RNA polymerase II (Pol II) is critical for most biological processes, including embryonic development. Transcription initiation occurs at the core promoter, frequently referred to as "the gateway to transcription". Core promoters are diverse in their architecture and function, and can contain distinct DNA motifs termed core promoter elements. Interestingly, downstream core promoter element (DPE)-containing genes are highly enriched for heart-related and mesodermal development GO terms. Unfortunately, the analyses of endogenous core promoter function *in vivo* within the developing embryo are largely lacking.

The *tinman* gene encodes a homeodomain-containing transcription factor that orchestrates the formation of the dorsal musculature and heart during *Drosophila* embryonic development. Using *in vitro* transcription and reporter assays, we previously showed that the *tinman* promoter contains a functional DPE motif. To address the *in vivo* importance of the DPE, we mutated the endogenous DPE of the *tinman* gene using the co-CRISPR approach. We show that the 7bp mutation of the DPE motif, located within the 5' UTR of the *tinman* gene, is sufficient to reduce *tinman* expression at both the RNA and protein levels.

Remarkably, significantly reduced numbers of *tinman*^{mDPE} flies are obtained when tested in trans to *tinman*^{null} allele, demonstrating the *in vivo* importance of the DPE. Although the heart is formed in mutant embryos, aberrant phenotypes are observed. Using multiple approaches, including nascent transcription dynamics during embryonic development, we provide mechanistic insights into the *in vivo* regulatory role of the DPE during Drosophila development. This discovery lays the groundwork for additional exciting findings related to transcriptional regulation of developmental genes via their core promoter.

516V **Identifying key drivers of gene regulatory networks in male *Drosophila*** Melissa Aldana, Angelica Aragon Vasquez, Claire Gray, Joseph L Aguilera, Mukulika Ray, Erica LarschanMCB, Brown University

Precise and coordinated regulation of gene expression during growth and development is essential for the viability of all organisms and to prevent diverse diseases ranging from cancer to neurodegeneration. Gene regulatory networks (GRNs) drive gene regulation during development and in response to environmental cues or therapeutics. However, the key drivers of gene regulatory networks are hard to predict. Therefore, I am disrupting an essential pioneer transcription factor in Drosophila called Chromatin-linked Adaptor for MSL complex (CLAMP) and identifying the GRNs that it regulates. CLAMP is critical in Drosophila dosage compensation, which is the two-fold upregulation of all the genes along the male X-chromosome; to achieve this upregulation, CLAMP recruits the Dosage Compensation Complex (DCC) to precise locations spread throughout the male X-chromosome. I performed Cleavage Under Targets and Release Using Nuclease (CUT & RUN) on wildtype and clamp mutant larvae to reveal the native and ectopic binding sites of CLAMP and the DCC. Concurrently, I performed mRNA-seq in all larvae to determine how dosage compensation was affected, specifically examining the male X-chromosome transcript output. My computational analysis using machine learning approaches will allow us to predict drivers of GRNs from CUT & RUN and mRNA-seq data. I will then compare the GRNs that I identify with those that have been identified for other critical transcription factors through the modENCODE project. Importantly, by understanding the fundamental mechanisms of gene regulation and regulatory networks, we can shed light on the complete effects of modern therapeutics.

517V **β-catenin/Armadillo mediated activation of Wg/Wnt target genes utilizes a biomolecular condensate dependent mechanism** Richard Stewart[1], Lauren Goodman[2], Jeannine Tran[2], Malavika Sabu[2], John Zientko[2], Kenneth Cadigan[21]MCDB, University of Michigan, [2]University of Michigan

Robust activation of Wg/Wnt target genes require the localization of β-catenin (called Armadillo in *Drosophila*), a transcriptional co-regulator, to Wnt-regulated enhancers (WREs). β-catenin/Arm is recruited to WREs through direct binding to TCF-family transcription factors and mediates transcriptional activation through binding to additional transcriptional co-activators. This process has traditionally been conceptualized through classic protein-protein interactions (e.g., Cadigan, 2008. Curr Biol 18: R943). It was recently reported that human β-catenin can form biomolecular condensates *in vitro* and *in vivo* (Zamudio, A, et al. 2019. Mol Cell 76: 753). They also identified aromatic residues in the N- and C-terminal intrinsically disordered regions (IDRs) of β-catenin that are required for condensation. We sought to extend these observations in mammalian and *Drosophila* systems. We have found that aromatic residues

in both the N- and C-terminal IDRs are roughly equivalent in importance for condensate formation. This is correlated with signaling activity of β-catenin in several functional assays using human cell culture. These results indicate that the N-terminal IDR has a previously unrecognized role in transcriptional activation (besides its well-established role in controlling β-catenin stability). Many of the aromatic residues within the IDRs are conserved in *Drosophila* and they are important for Arm's ability to regulate Wg target genes. However, the dependency varied in different contexts, e.g., an *arm* mutant transgene lacking aromatic residues in the IDRs is still capable of rescuing an *arm* loss of function mutant to a surprising degree. But in the developing eye, loss of aromatics in either IDR was sufficient to inactivate Arm's signaling activity. While these results support a model for the ability of β-catenin and Arm to form condensates is linked to its ability to activate Wg/Wnt targets, it is also possible that mutation of aromatic residues interrupt binding to essential co-activators. To address this point, we took advantage of our findings that β-catenin /Arm lacking the N-terminal IDR was deficient in condensate formation/biological activity. We then rescued these properties by fusing heterologous IDRs to β-catenin /Arm. These "IDR swap" results solidify our working model that condensate formation is inextricably linked to transcriptional activation of Wg/Wnt targets.

518V **Role of *drumstick* to specify the anterior-most domain of *Drosophila* hindgut.** Sarder UddinCity Colleges of Chicago

The gene *drumstick (drm)* is necessary for specifying the small intestine, an anterior domain of ectodermal hindgut of *Drosophila melanogaster*. However, mechanisms that specify the small intestine as well as gene regulatory pathways leading to transcriptional activation of *drm*, are still unclear. In this investigation, it has been found that *drm* expressed in the regions abutting anterior end of the hindgut primordium, which is posterior-most region of the endoderm and in basal portion of Malpighian tubules, but not in prospective region of small intestine. The small intestine failed to form in mutant embryos that lack both the endoderm and Malpighian tubules, but was allowed to develop if either one of the *drm*-expressing tissues remained intact. This result indicates that *drm* induces the development of small intestine cell non-autonomously, probably by activating some intracellular signaling. Several Wnt family genes also expressed in the border region between hindgut and endoderm, but signaling triggered by *drm* were independent of canonical Wnt pathway, since small intestine developed normally in mutant deficient for armadillo (β-catenine). Regarding regulation of *drm* expression, it also been found that *drm* expression in the posteriog gut region disappears in *tll* embryos, and, small intestine failed to develop. On the other hand, forced-expression of *tll* caused expansion of the *drm* expression in posterior gut region, resulting in an expanded small intestine. This results demonstrate that *drm* is activated under control of *tll*, and consequently *drm* act on hindgut primordium cell non-autonomously to specify the small intestine.

519T **Probing the chromatin landscape of a repetitive locus using DiMeLo-seq** Thomas OHaren, Leila RiederBiology, Emory University

Histone proteins are critical for organizing the genome, and precise regulation of their expression is essential during development. Histone levels are especially important in the early embryo when cells divide rapidly. The canonical histone genes cluster in the genome as tandem, repetitive units (collectively called the histone locus) to allow for quick and precise regulation. Epigenetic signatures of the genome, such as histone modifications, emerge during early embryogenesis and contribute to the control of gene expression in these earliest rounds of cell division. However, the epigenetic landscape of the histone locus is unknown as the repetitive nature of the histone genes makes it difficult to analyze chromatin characteristics using traditional short-read techniques, such as ChIP-seq. The newly developed DiMeLo-seq (Directed Methylation with Long-read sequencing) circumvents this issue by utilizing antibody-directed DNA methylation as opposed to immunoprecipitation in order to probe regions of protein-DNA interactions. This allows for the capture and sequencing of long-reads (~10-20kb) that contain sufficient, unique sequence to map to even highly repetitive regions of the genome like the histone locus. Using antibodies targeting transcriptional machinery and well-studied histone modifications involved in epigenetic control of gene expression, the chromatin landscape of the histone locus can be revealed. Mapping H3K9me3, a marker of heterochromatin typically found at centromeric regions, from *Drosophila* embryos to the histone locus reveals how spreading of the mark may regulate histone gene expression and may explain the presence of over 100 copies of each replication dependent histone gene in the genome. By performing DiMeLo-seq targeting phosphorylated RNA polymerase II, we can determine which genes of the locus are transcriptionally active. Overall, DiMeLo-seq reveals the epigenetic landscape of previously unmappable regions to better understand how these marks control gene expression of repetitive regions in the early embryo.

520T **A ZAD zinc finger protein guides installation of a mini H3K9me3 silencing domain to secure female germ cell identity** Helen SalzCase Western Reserve Univ

The H3K9me3 histone modification has well-characterized roles in heterochromatin formation and transposable element (TE) silencing. H3K9me3 chromatin also silences protein coding genes essential for cell fate maintenance. How the machinery responsible for H3K9 methylation finds its target genes is poorly understood. Here we explore this question in *Drosophila* female germ cells where H3K9 methylation secures sexual identity by silencing male-specific *phf7* transcription. Of the three enzymes known to methylate H3K9, only SETDB1 (encoded by *eggless*) plays a role in silencing *phf7*. SETDB1 also functions in piRNA-guided TE silencing. Contrary to expectations, the dedicated piRNA pathway components, *piwi, aub, rhino, panx,* and *nxf2,* are not required for *phf7* silencing. Thus, the mechanisms controlling H3K9me3 deposition onto *phf7* and TEs are distinct. In mammals, SETDB1 can be recruited to its targets by members of the KRAB-zinc finger family of sequence-specific DNA binding proteins. Although the KRAB family is confined to mammals, it has been hypothesized that members of the insect-specific ZAD zinc finger protein family are functional analogs. The ZAD zinc finger protein family arose in the ancestor of arthropods and vertebrates, expanding to become the most abundant class of transcription factors in *Drosophila* and related species. Most of the ZAD zinc finger proteins, however, are uncharacterized. We, therefore, carried out a targeted RNAi screen and discovered that CG4936 is required for *phf7* silencing in female germ cells. Loss of *CG4936* in germ cells interferes with H3K9me3 deposition and *phf7* transcriptional regulation, leading to ectopic PHF7 protein expression. We, therefore, named CG4936 IDENTITY CRISIS (IDC). IDC is a 521 amino acid (aa) protein with an array of 5 C2H2 zinc fingers at its C-terminal end (386-491 aa), suggesting sequence-specific DNA binding activity. In agreement, we find that a functional IDC-GFP fusion protein localizes to the conserved non-coding first exon of *phf7* in ovarian extracts. Collectively, our data establish that the DNA binding protein IDC directs the H3K9 methylation machinery to build a silencing domain at the *phf7* locus, thereby preventing accidental female-to-male programming.

521T **Determining essential, pioneering features of the conserved transcription factor Grainy head** Meghan Freund, Tyler Gibson, Andrew Rashoff, Peter Lewis, Melissa HarrisonUniversity of Wisconsin-Madison

Multicellular organisms are made up of many differentiated cell types that perform unique functions. Beginning from a single cell, organisms undergo a journey of differentiation, forming a variety of unique cell types. Because these cells all possess the same genome, distinct cell types are not due to differences in genotype, but rather differences in gene expression. Transcription factors bind DNA and drive this differential gene expression. However, compacted chromatin acts as a barrier to transcription-factor binding. A specialized class of transcription factors, known as pioneer factors, can overcome this barrier by binding to condensed chromatin and increasing chromatin accessibility. While pioneer factors share the ability to bind condensed chromatin, the mechanisms governing their unique binding profiles are not well understood. Grainy head (Grh) is an essential pioneer factor that drives epithelial cell fate and when mis-expressed, can lead to cancer. It is conserved across species and binds the same canonical DNA sequence in all species studied. These features make Grh a great candidate to elucidate the basic mechanisms by which pioneer factors engage the genome and their role in shaping cellular identity. We combined *in vitro* and tissue-culture assays to examine how Grh finds its motif within compacted chromatin and promotes accessibility. We showed that full-length Grh, but not the DNA-binding domain (DBD) is able to bind nucleosomes and drive accessibility. Thus, regions outside of the DBD promote nucleosome binding. Additionally, we are investigating how the positioning of the canonical sequence on the nucleosome affects binding. Like a subset of pioneer factors, Grh is retained on the mitotic chromosomes. The features that enable this retention and the functional significance remain unclear. In contrast to the pioneering activity of Grh, the DBD is sufficient for mitotic retention and residues that mediate DNA binding are necessary. Because the DBD, but not regions outside, are conserved in mammals, we propose that mammalian Grh orthologs may also be retained on mitotic chromosomes and are currently testing this. In addition, to understand what features allow Grh to engage closed chromatin we are determining the regions of the N-terminus required for chromatin binding *in vivo* and *in vitro*. Together, our data will determine how Grh scans the genome, recognizes its motifs within inaccessible chromatin, and drives transitions in cell fate.

522T **A Dual-activity Topoisomerase Interacts with piRNA Machinery to Promote Transposon Silencing and is needed for Germ Cell Functions in *Drosophila* ovary** weiping shen[1], Seung kyu Lee[2], William wen[2], yutong xue[2], tiangyi zhang[2], shuaikun su[2], yongqing zhang[2], alexei sharov[2], Weidong Wang[21]National Institute on Aging, [2]NIH

Topoisomerase 3b (Top3b)-TDRD3 is the only dual-activity topoisomerase complex in animals that can change topology for both DNA and RNA; and has been implicated in DNA transcription, mRNA translation, and siRNA-guided heterochromatin formation and transposon silencing. Human and mice carrying mutations in Top3b or TDRD3 have been associated with fertility and germ cell disorders, but the underlying mechanism remains unclear. Here, we show that Top3b-TDRD3 localizes mainly in the cytoplasm and stably associates with several components of the piRNA pathway in

mouse and fly germ tissues. Moreover, *Drosophila* mutants of Top3b-TDRD3 exhibit germ cell defects similar to those of piRNA pathway mutants, including de-silencing of transposons, defective piRNA biogenesis, impaired oogenesis, and reduced fertility. Furthermore, Top3b-TDRD3 acts in both germ and somatic cell piRNA pathways to silence transposons. Notably, *Top3b* exhibits strong genetic interactions with several piRNA biogenesis enzymes, including helicases and nucleases, to promote piRNA biogenesis, transposon silencing, and oogenesis. Our data reveal a novel role for a topoisomerase in RNA metabolism—working with piRNA biogenesis enzymes to produce piRNAs; and suggest a mechanism of how Top3b-TDRD3 mutations can lead to germ cell and fertility disorders.

523T **_D. melanogaster HP1b/HP1c_ double-mutants are viable and fertile with only minor changes in HP1a localization** Sarah K Sims, Nicole C RiddleBiology, University of Alabama at Birmingham

Heterochromatin Protein 1 (HP1) proteins are an important protein family involved in the maintenance of chromatin states. HP1 proteins can form homo- and heterodimers, which bind to other chromatin elements such as histones, DNA, and a variety of protein partners. HP1 proteins are essential for ensuring the safety and functions of the genome and are highly conserved in eukaryotes. The genome of *Drosophila melanogaster* contains three somatically expressed HP1 genes: *Su(var)205* encoding HP1a, *HP1b*, and *HP1c*. Loss of each of the three proteins has important consequences, with mutations leading to the misexpression of hundreds of genes and decreased viability and/or fertility. When mutations in HP1 proteins are studied, the impact on the other HP1 family members typically is not assayed, despite them occurring together in protein complexes. Here, we investigate how HP1 proteins interact by examining double-mutants lacking HP1B and HP1C. We find that double-mutant flies are viable and healthy, as their fertility and embryo hatch rate is similar to a control stock. Polytene chromosome analysis in these *HP1b/HP1c* mutants suggests that while the remaining HP1 homolog HP1a continues to generally be enriched at the centromeres, there are subtle changes in its localization in the double-mutants. Ongoing studies focus on how loss of HP1B and HP1C affects gene expression and chromatin structure genome-wide using next generation sequencing methods. Although double-mutants have normal development and fertility, RNA-seq gene expression analysis shows significant differences in gene expression between control and *HP1b/HP1c* double-mutant flies. Together, our data demonstrate that complete loss of HP1B and HP1C is survivable in *D. melanogaster* and leads to only minor changes to HP1a localization. Our study highlights possible crosstalk and cooperative functions of HP1 proteins and has the potential to provide further insights into the functions of the sole remaining HP1 protein, HP1a.

524T **The function and evolution of _abnormal oocyte,_ a _Drosophila_ histone regulator** Risa Takenaka[1,2], Harmit S Malik[3,41]Molecular and Cellular Biology, University of Washington, [2]Fred Hutchinson Cancer Center, [3]Division of Basic Sciences, Fred Hutchinson Cancer Center, [4]Howard Hughes Medical Institute

Histones are the core unit of chromatin that package eukaryotic genomes. Insufficient histone levels result in poor packaging and promiscuous transcription, whereas excess histone levels impede transcription and other essential cellular processes. Therefore, an optimal histone-to-genome ratio is critical to maintain cellular functions and organismal fitness.

The *abnormal oocyte* (*abo*) gene in *Drosophila* encodes a repressor of core histones. Larry Sandler isolated *abo* in 1968 and identified its maternal-effect lethal phenotype. *abo* homozygous-mutant females produce fewer offspring relative to their heterozygous sisters. This phenotype results from an overproduction of histones in *abo* homozygous-mutant females, which disrupt the maternal-to-zygotic transition of gene activation during embryogenesis.

Despite *abo'*s important function, *abo*-mutant adults show no other morphological phenotypes. Therefore, the *Drosophila abo* is an intriguing model for studying novel aspects of tissue-specific vulnerabilities to histone overexpression. To this end, I generated flies with a CRISPR/Cas9-mediated knockout of *abo* to accurately interpret the *in-vivo* consequences of histone overexpression. I then used RNA-seq to investigate whether the soma and germline vary in their responses to histone overexpression.

In parallel, I used the McDonald-Kreitman test and found evidence of positive selection having shaped the evolution of *abo* between *D. melanogaster* and *D. simulans* (NI=0.485, *p*=0.018). I hypothesize that this rapid evolution may have resulted in a species-specific histone regulation by *abo*. To test this hypothesis, I complemented my CRISPR/Cas9-mediated knockout of *abo* in *D. melanogaster* using a transgene rescue *abo* gene from *D. simulans*. Using these flies, I will test whether *D. simulans* Abo is able to regulate *D. melanogaster* histones, and as a result, rescue the maternal-effect lethal phenotype. Finally, I will determine Abo's binding site(s) for con- and hetero-specific *abo* using CUT&Tag to evaluate how positive selection may have altered its DNA-binding specificity.

525T **Transcript-specific effects of developmental ethanol exposure on the expression of chromatin-modifying genes in Drosophila** Joshua A Marsh, Jodi Nguyen, Sanjana Anam, Rohit Radhakrishnan, Rachael FrenchBiology, San Jose State University

Ethanol is a teratogen. Developmental alcohol exposure (DAE) in humans leads to a Fetal Alcohol Spectrum Disorder (FASD). Individuals with FASD can exhibit a variety of deleterious phenotypes, including slow growth, metabolic changes, behavioral difficulties, and intellectual disabilities. Recent findings in mammals indicate that metabolic changes associated with DAE involve long-term changes in gene expression mediated by epigenetic effects.

We have established *Drosophila* as a model for FASD. Using this model, we have found that flies exposed to ethanol during larval stages display phenotypes similar to mammals exposed during fetal development, including developmental delay, reduced adult size, smaller brains, CNS dysfunction, reduced sensitivity to ethanol sedation, impaired insulin signaling and lipid metabolism, and reduced survival. Some of these phenotypes, including impaired lipid metabolism and sedation resistance, persist into adulthood. Consistent with this observation, we have shown, using quantitative RT-PCR, that DAE causes long term changes in gene expression.

Ethanol exposure in adult flies alters the expression of histone modifying enzymes. We hypothesize that some of the long-term changes in phenotype and gene expression caused by DAE are due to epigenetic alterations in gene expression due to similar effects on the expression of histone modifiers. To test this hypothesis, we used survival assays to test the DAE sensitivity of flies mutant for a variety of histone modifiers. In addition, we used qPCR to measure the expression of genes encoding histone modifiers in ethanol-reared larvae.

We will present data showing that mutations disrupting the histone modifiers Sirt1, Lid, dG9a, JHDM2, NO66, and Nejire (Nej) result in changes in sensitivity to DAE. Additionally, DAE causes reduced expression of G9a, Sirt1, and lid in larvae, and specifically upregulates at least one nej transcript. Thus, DAE alters epigenetic regulation of gene expression, and it is likely that some DAE-induced phenotypes are due to these effects.

We will additionally present the results of experiments to test the effects of DAE on additional histone modifying enzymes, as well as the role of those proteins in sensitivity to developmental ethanol. We are also testing whether persistent DAE-induced phenotypes are due to changes in epigenetic regulation of gene expression. This research was supported by grants from the National Institutes of Health National Institute of General Medical Sciences (5SC3GM103739) and National Institute on Alcohol Abuse and Alcoholism (1R15AA027678), and a Project Development Grant from the California State University Program for Education and Research in Biotechnology.

526T **Determining how "reader" proteins interact with the genome: Does L(3)mbt require methylation of histone H4 lysine 20 to bind chromatin?** Megan B Butler[1], Aaron T Crain[2], Robert J Duronio[11]Biology, University of North Carolina at Chapel Hill, [2]University of North Carolina at Chapel Hill

Generation and maintenance of specific chromatin domains are essential for proper genome regulation, cell cycle progression, and organismal development. These domains are created and modulated by chromatin "reader" proteins. Reader proteins bind to the post-translationally modified N-terminal tails of histone proteins and act either directly or as part of multi-protein complexes to alter chromatin structure and control critical genome functions. The *Drosophila melanogaster* Lethal (3) malignant brain tumor (L(3)mbt) protein functions as a transcriptional regulator in a variety of tissues throughout development, and mutation of *l(3)mbt* causes tumors in larval brains. L(3)mbt and its human homolog L3MBTL1 have also been implicated in chromatin condensation via their MBT domains. The MBT domains of L(3)mbt are thought to specifically recognize histone H4 lysine 20 mono- and di-methylation (H4K20me1/2) based on *in vitro* peptide association data. However, *in vivo* studies suggest that L(3)mbt does not exclusively co-localize with H4K20me1/2 throughout the genome; thus, how L(3)mbt interacts with chromatin and controls gene expression remains incompletely understood. Determining whether L(3)mbt depends on H4K20me for binding the genome *in vivo* has never been addressed in the most direct way – by mutating H4K20. We generated H4K20R mutant animals to assess the consequence of eliminating H4K20me on the association of L(3)mbt with the genome. We GFP-tagged *l(3)mbt* at the endogenous locus using CRISPR-Cas9 and the Scarless Gene Editing system. This allele can be maintained as a homozygote in which all L(3)mbt molecules are GFP-tagged. Using fluorescent confocal microscopy of whole mount tissue, we detected a small decrease in total nuclear GFP-L(3)mbt signal in H4K20-mutant animals without any detectable change in protein localization. To assess L(3)mbt's interaction with the genome at a higher resolution, we used the *in situ* genomic profiling assay CUT&RUN. In a wild-type background, the strongest peaks of GFP-L(3)mbt are located at the transcriptional start site of genes, whereas H4K20me1 peaks are enriched at gene bodies. Accordingly,

only 40% of GFP-L(3)mbt peaks overlap with previously generated modENCODE H4K20me1 ChIP-sequencing peaks. These data suggest that L(3)mbt does not largely depend on H4K20me for its interaction with chromatin, a hypothesis we will test by assessing GFP-L(3)mbt genomic occupancy via CUT&RUN in H4K20R mutant animals.

527T **Consequences of activating a centromere-enriched retroelement** Tyler McDermott[1,2], Barbara Mellone[1,21]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

Retroelements are a class of selfish genetic elements capable of transposing in the genome via the reverse transcription and re-insertion of their RNA product. Despite the deleterious effects that their activity has on genomic stability, retroelements have been proposed to play a role in establishing centromere identity. Centromeres are the essential chromosomal loci for kinetochore assembly, and thus, chromosome segregation. Mistakes in centromere functionality lead to chromosome missegregation events, and thus, genomic instability. Therefore, the notion that a "genetic parasite" could persist at such essential regions as centromeres is peculiar, though not rare. Retroelements have been found enriched at the neocentromere of human cell line 10q25, at every evolutionarily new centromere of the donkey, and at every centromere in *Drosophila melanogaster* and *simulans*, as well as in several other species from across taxa. Recent work from our group showed that every centromere of *Drosophila melanogaster* contains at least one copy of the same retroelement, *G2/Jockey-3.* This association begs questions as to whether this element may be functionally linked to CENP-A, the epigenetic mark for centromeric chromatin. *G2/Jockey-3* could preferentially insert within CENP-A chromatin, or provide a favorable environment for biased deposition of CENP-A. To test these possibilities, we designed a transgenic fly line containing an engineered, full-length copy of *G2/Jockey-3*(*eJockey-3*) that can be activated in progeny by selective mating and whose transposition can be tracked in the genome. Preliminary experiments testing the effects of *eJockey-3* activation on fly viability and fertility show deleterious phenotypes suggesting genomic instability. While we did not observe abnormal karyotypes in tissues expressing *eJockey-3*, we did detect DNA damage biasing the centromere by immunofluorescence microscopy (IF) which may be attributed to the uncharacterized endonuclease encoded within the retroelement. Using custom digital droplet PCR (ddPCR) assays, we detected copy number variation of *eJockey-3*, suggesting active transposition. Further experiments are currently underway to determine if, and how, *eJockey-3* may be affecting the centromere. These include specialized library preps and chromatin immunoprecipitations for centromeric DNA. We are also generating transgenic lines for characterizing the protein products of *eJockey-3* via epitope tagging and ORF mutations.

528T **Evolutionary conservation of chromatin-modifying and insulator sequences in Drosophila** Alia Khogali[1], Miki Fujioka[2], James B. Jaynes[2], Hemlata Mistry[11]Widener University, [2]Thomas Jefferson University

even skipped (*eve*) is a conserved regulator of developmental processes important for pattern formation. Because pattern-forming genes are conserved over vast evolutionary distances, we can dissect these genes in model organisms to identify those DNA sequences that interact with the molecular machinery of the cell to control gene expression in discrete temporal and spatial domains. Genomic functional elements, including those affecting chromatin conformation and the spread of heterochromatin, have been identified in *D. melanogaster* that contribute to the dynamic changes in *eve* expression during development. At the 3' end of the *eve* locus, upstream of the adjacent gene, is a Polycomb Response Element (PRE) flanked by an insulator sequence. Together, these chromatin-modifying sequences contribute to the dynamic changes in *eve* expression during development. To understand if similar insulator and chromatin-modifying functions have been conserved during evolution in related species, we examined the corresponding genomic regions from three related *Drosophila* species: *D. erecta*, *D. pseudoobscura*, and *D. virilis*. In *D. erecta*, which is most closely related to *D. melanogaster*, *eve* is linked to *TER94*. In the more distantly related species, *D. pseudoobscura* and *D. virilis*, *eve* is adjacent to *Usp15-21 (CG30421)*.

Insulator sequences can interact with each other to form topologically associating domains in DNA. The PRE-insulator fragment from *D. melanogaster* has been shown to be capable of facilitating enhancer-promoter interactions in *trans* by homologous pairing (transvection). We cloned the genomic fragments homologous to the *D. melanogaster* PRE-insulator region from the three related species into both a reporter transgene and an enhancer transgene. After generating transgenic stocks via phiC31-mediated recombination in *D. melanogaster*, we determined whether there is transvection-mediating activity within the homologous PRE-insulator fragment from a single species, and if there are interactions among the fragments from different species. We observe different intensities of reporter expression depending on the extent of functional conservation. We are in the process of further dissection of the functional sequences. Our comparative approach using these four *Drosophila* species will give us an understanding of how the homolog pairing

function of PREs and insulators has changed during evolution, and an inroad to the mechanistic basis of those changes.

529T **Is X recognition dependent on nuclear architecture?** Maggie SneidemanWayne State University

Drosophila melanogaster males carry one X and one Y chromosome, but females have two X chromosomes. To equalize expression of X-linked genes between the sexes, males increase transcription of X-linked genes approximately two-fold. Increased expression involves the Male-Specific Lethal (MSL) complex, which modifies chromatin. The MSL complex first binds a motif found in Chromatin Entry Sites (CES) on the X, and spreads into nearby genes through binding to an active histone mark. However, this mark, and the motif in the CES, are also found throughout the genome but fail to recruit the MSL complex to autosomal sites. Another factor must therefore distinguish the X from the autosomes. The X chromosome is strikingly enriched for chromosome-specific repeats. One of these is the AT-rich 1.688^{X} repeats. Our lab has previously shown that the 1.688^{X} repeats play a role in identifying the X. The focus of my project is to identify non-histone proteins that could participate in X identification by binding to 1.688^{X} repeats or modulating architecture of the X chromosome. We tested proteins known to bind satellite DNA or with AT hook motifs, proteins with X-specific or male-specific phenotypes, and heterochromatin factors. Knock down of candidate genes was used to test for a male specific phenotype. A strain with compromised X recognition was used to determine genetic interaction with each candidate gene, revealed by reduced male survival. I also used an autosomal reporter for MSL recruitment to differentiate recruitment by the CES and 1.688^{X} repeats. As a proof of concept, we determined that *ISWI*, a gene with male biased mutant lethality and selective disruption of the polytenized male X, selectively reduces male survival in the strain with compromised X recognition. Our studies also identified satellite-binding proteins and factors known to structure chromatin as factors in X recognition. These studies suggest that repeat binding proteins may collaborate with 1.688^{X} satellites to promote recognition of X chromatin.

530T **Identifying limitations to pioneer factor-mediated reprogramming** Eliana F. Torres-Zelada[1], Elizabeth D. Larson[1], Hideyuki Komori[2], Zoe Fitzpatrick[1], Cheng-Yu Lee[2], Melissa M. Harrison[11]Department of Biomolecular Chemistry, University of Wisconsin School of Medicine and Public Health, [2]Department of Cell and Developmental Biology and Life Sciences Institute, University of Michigan

Transcription factors coordinate changes in cellular identity by binding DNA and driving gene expression. However, chromatin is barrier to transcription-factor binding. Pioneer factors are a class of transcription factors that overcome this barrier by binding DNA within nucleosomes, establishing regions of accessible chromatin, and promoting binding of additional factors to regulate gene expression. These properties make pioneer factors instrumental in driving developmental transitions. We and others showed that the pioneer factor, Zelda (Zld) is required for the reprogramming that takes place following the unification of the sperm and the egg at fertilization. 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 NB and embryos. Indeed, other pioneer factors show cell-type-specific DNA binding. Thus, while pioneer factors can bind to DNA within nucleosomes, there are features that shape this binding. We are using Zld binding in the embryo and NB as a model to understand those features that promote and repress pioneer-factor binding. Zld is a 1596 amino acid protein that includes six zinc fingers motifs of which four (ZnF3-6) are required for DNA binding. We previously demonstrated that ZnF2 acts as an auto-inhibitory domain in embryos and cell culture. Unexpectedly, when we assayed the functional requirement of these motifs in the NBs, we showed ZnF2 is necessary for promoting the stem-cell fate. We demonstrated that ZnF2 is required to promote DNA binding in NB, but not the embryo. Indeed, Zld with mutations in the canonical DNA-binding domain was still capable of binding broadly in the NB. These data suggest that ZnF2 is specifically required in the NB to promote genome occupancy. In S2 cells, Zld binding and pioneering at sites harboring the canonical Zld-binding motif require the canonical DNA-binding domain. By contrast, sites that do not possess the canonical motif require ZnF2 for binding. Mutations in ZnF2 promote Zld binding at loci containing the canonical Zld-binding motif. Together, our data suggest that ZnF2 functions to stimulate Zld binding at regions of the genome that do not possess the canonical motif, and we are currently testing whether this is mediated directly by DNA binding or by potentiating interactions with specific cofactors. Ultimately, our work is uncovering novel mechanisms to regulate pioneer-factor occupancy.

531T **Targeting an Active Chromatin Domain to the X-chromosome** Claire Gray, Joseph Aguilera, Melissa Aldana, Angélica Aragón Vásquez, Mukulika Ray, Erica LarschanDepartment of Molecular Biology, Cell Biology, and Biochemistry, Brown University

Transcription must be tightly regulated to drive normal organismal development and to prevent the formation of

disease states from cancer to neurodegeneration. To coordinate the regulation of genes, chromatin domains are formed to concentrate key factors at discrete genomic loci that activate or repress sets of genes. Pioneer transcription factors–which have the ability to bind to closed chromatin, recruit chromatin remodelers to open chromatin, and target additional transcription complexes–play a significant role in generating active and repressive chromatin domains. Using the male Drosophila dosage-compensated X-chromosome as a model, I am investigating how protein-binding domains of the genome-wide pioneer transcription factor, Chromatin Linked Adaptor for MSL Proteins (CLAMP), functions to specifically target an active chromatin domain to the X-chromosome. I hypothesize that mutations in CLAMP protein-binding domains will alter proper chromatin domain formation, resulting in the misregulation of dosage compensation. First investigating CLAMP genome-wide targeting, I performed Cleavage Under Targets and Release Using Nuclease (CUT&RUN) on wildtype and clamp mutant larvae to reveal the role of CLAMP protein-binding domains in its global binding. Concurrently, I checked the functionality of dosage compensation in all larvae by performing mRNA-seq, specifically monitoring for significant changes in the X-chromosome transcript output. This study will reveal the importance of specific CLAMP protein-binding domains in chromatin domain formation, which is critical in robust dosage compensation. Subsequently, I will perform Hi-ChIP to define the three-dimensional chromatin interactions mediated by CLAMP, shedding a complete light on the basic mechanisms of chromatin domain formation.

532T **Determining functional differences between canonical H3.2 and variant H3.3 during *Drosophila* development** Jeanne-Marie E McPherson[1], Robert E Duronio[2], Daniel McKay[21]Genetics, University of North Carolina at Chapel Hill, [2]University of North Carolina at Chapel Hill

Histone proteins package and organize DNA into chromatin, which regulates all DNA-dependent processes. The regulation of histone abundance and histone type is critical, as too many or too few histones is toxic to cells and disrupts development. Cells contain two histone types: canonical histones that are expressed during S phase of the cell cycle, 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. The high conservation of amino acid differences between canonical H3.2 and variant H3.3 suggests that each histone type may perform unique functions in the genome, yet it is not understood if variant H3.3 function is mediated by its cell-cycle independent expression or its unique protein sequence. Here, we generated *Drosophila* CRISPR mutants that express H3.2 from the endogenous *H3.3* genes ($H3.3^{H3.2}$) and examined the roles of H3.3 in development, fertility, and gene regulation. We find that H3.3 is required for male and female fertility; *H3.3A* is essential for male fertility, while female fertility requires expression of *H3.3A* or *H3.3B*. These data indicate that the unique protein sequence of H3.3 is required for fertility, rather than only replication-independent H3 expression. Strikingly, $H3.3^{H3.2}$ females have severely reduced viability, but males are fully viable. Most $H3.3^{H3.2}$ females die at the pupal stage. To our knowledge, this is the first report of female-specific requirements for H3.3. We are now characterizing which amino acids unique to H3.3 are required for its specific functions. We have focused on residue S31 in the tail of H3.3, which is otherwise identical to the tail of H3.2. We have generated $H3.3^{S31A}$ mutants to determine whether loss of S31 is solely responsible for $H3.3^{H3.2}$ phenotypes. Additionally, we found that H3.3 is essential for development when H3.2 gene copy number is reduced, suggesting a previously unknown requirement for coordination between H3.2 and H3.3 expression. To identify genes required for this coordination, we conducted a genetic screen which identified histone H3 dose as a modifier of *Polycomb* (*Pc*) function. Specifically, loss of H3.3 enhances *Pc* mutant phenotypes. We are continuing this work by examining the effects of $H3.3^{H3.2}$ and $H3.3^{S31A}$ mutations on *Pc* function. Our work is revealing the specific requirements and crosstalk between canonical and variant histone H3 throughout development.

533F **Deciphering developmentally regulated DSB repair outcomes at single allele resolution** Zhiqian Li[1], Dave Kosman[2], Ethan Bier[21]Cell and Developmental Biology, University of California, [2]University of California

Living cells resolve double-stranded breaks (DSBs) through a sophisticated process with multiple cellular repair pathways, while the choice of a particular pathway is determined by a combination of factors including cell cycle phase, genomic context, and nature of target DNA sequences. Little is known regarding how this complex decision process is regulated during development of a multicellular organism. Here, we develop the Integrated Classifier Pipeline (ICP) with two linked and recursive DSB classifiers: 1) a nucleotide-position based DSB classifier (NPClassifier), and 2) a single allele resolution based DSB classifier (SAClassifier) to parse Cas9-targted chromosomal DSBs in both *Drosophila melanogaster* and malaria mosquito vector *Anopheles stephensi*. These studies uncover a dynamic and developmentally-associated DSB repair switch, with early lesions (during pre-blastoderm stage) being repaired primarily through non-NHEJ (non-homologous end joining, including microhomology-mediated end joining MMEJ and insertion), while later generated DSBs being predominantly repaired by canonical NHEJ (c-NHEJ). We also provide evidence for particular ICP utilities at:

deciphering highly reproducible locus and lineage-specific somatic mutation profiles, tracking developmental stage-dependent generation of individual recurrent indels and transgenerational transmission of specific alleles, and following copying of gene cassettes via homology directed repair (HDR) in a marker-free manner using PCR/NGS-based methods. ICP is a powerful analytic tool for integrating interpretation of diverse DSB repair outcomes including NHEJ and HDR events within the same sample. These studies highlight the power of ICP platform to resolve and track developmentally regulated and lineage dependent DSB repair outcomes in diverse settings.

534F **The motif-1 interacting protein (M1IP) colocalizes with CP190 and M1BP near TAD borders** Dagyeong Yang[1,2], Elissa P. Lei[11]NIDDK, NIH, [2]University of Maryland College

The interphase genome is organized in three-dimensional space at various levels, including compartments, territories, and topologically associated domains (TADs). Active transcription is observed at many TAD borders, and various chromatin insulator proteins are also enriched at these locations, particularly CP190. The core promoter motif-1 is enriched at these sites, and the transcription factor M1BP that binds to motif-1 is also present. It was recently shown that both CP190 and M1BP physically interact and are both required for motif-1 dependent gene expression and transcription near TAD borders. In this study, we evaluate a novel Motif-1 interacting protein (M1IP), which was previously identified as a motif-1 binding protein that was copurified along with CP190 and M1BP. M1IP contains a BED-type zinc finger domain also found in the BEAF-32 insulator protein. Using ChIP-seq data produced by the ENCODE project, we found that M1IP overlaps extensively with CP190, M1BP, and BEAF-32. Using MEME-ChIP, we verified that motif-1 is enriched at M1IP chromatin binding sites across the genome. Additionally, we found that M1IP is also highly enriched at TAD borders. Current studies are underway to elucidate the possible function of M1IP in transcription regulation by performing RNA-seq after depleting M1IP. We will also assess whether M1IP physically interacts with M1BP and/or CP190.

535F **How chromatin state affects mitotic recombination rates** Priscila Santa Rosa[1], Robert J. Duronio[2,2,3,4,5], Jeff Sekelsky[2,4,41]The Curriculum Genetics and Molecular Biology, University of North Carolina at Chapel Hill, [2]Department of Biology, University of North Carolina at Chapel Hill, [3]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, [4]Integrative Program for Biological and Genome Sciences, University of North Carolina at Chapel Hill, [5]Department of Genetics, University of North Carolina at Chapel Hill

Mitotic recombination (MR) results from a DNA repair mechanism in which homologous chromosome arms are exchanged during mitosis. Although homologous recombination is a critical event in meiosis and essential for DNA repair, MR can result in undesirable outcomes, such as loss of heterozygosity (LOH). In this event, an individual who is heterozygous for one functional and one nonfunctional allele has cells that become homozygous for the nonfunctional allele. One classical example of LOH is the loss of the retinoblastoma tumor suppressor gene *RB1*, leading to the development of a malignant retinal tumor in the eye of infants. MR occurs in a context of chromatinized DNA, but it is unknown whether chromatin state affects the frequency of these events. Therefore, we hypothesize that different chromatin environments alter MR frequency. I will test this hypothesis using three approaches. First, we are investigating whether specific histone residue mutations cause MR in the male germline (MR does not occur in the male germline). We are using flies from the histone gene replacement platform, containing mutated histone genes together with deletion of the endogenous histone gene locus, to build males in which MR can be analyzed by looking at recessive markers on the 3rdchromosome. In the second aim, we are using the MARCM technique to detect MR and label cells in larval wing discs, both in wild-type larvae and in larvae with the same histone mutations. This will enable us to map recombination sites to identify potential hotspots or chromatin marks associated with MR. Preliminary result shows that a reduced number of histone genes (12 copies instead of 100 copies of each histone gene) does not cause MR in male germline cells. In the next step we will test whether different mutants alter MR frequency. One example is H3K9 which is a marker of heterochromatin when trimethylated. We expect that changing lysine to arginine (H3K9R), which cannot be methylated, will disrupt heterochromatin and potentially induce MR. In conclusion, this system will enable us to identify histone mutations that can affect the rate of MR and regions in the fly genome that are more prone to recombination.

536F **Impacts of the epigenetic silencing of transposable elements on local recombination rate** Yuheng Huang[1], Zita Gao[1], Kevin Wei[2], Grace Yuh Chwen Lee[11]UC-Irvine, [2]UC-Berkeley

A negative association between meiotic recombination rates and transposable elements (TEs), the widespread genomic parasites, has been widely observed across taxa. While the field usually interprets such association as the results of reduced efficacy of selection against TEs in regions of low recombination, direct modification of recombination rates

by TEs is another potentially potent, but largely unexplored, possibility. One mechanism by which TEs impact local recombination rates is through altering chromatin environments. To reduce the selfish replication of TEs, hosts have evolved mechanisms to epigenetically silence euchromatic TEs through the deposition of heterochromatic marks, mainly H3K9me2/3. Because the enrichment of heterochromatic marks has been widely associated with suppressed recombination, we hypothesize that the TE-mediated enrichment of heterochromatic marks results in reduced local recombination rates in the euchromatic regions. To test the hypothesis, we studied the associations between the distribution of crossover events and epigenetically silenced TEs in two inbred lines with distinct TE insertion profiles. We developed a novel approach that uses long-read PacBio sequencing to efficiently identify crossover events among pooled recombinant individuals. Such an approach mitigates the need for sequencing individual flies in the traditional method and can be easily scaled up to uncover a large number of crossover events. We benchmarked our approach by comparing a panel of backcrossed offspring that were sequenced individually with Illumina and as a pool with PacBio long-reads. According to the benchmark data, our approach has a low false positive rate (6.5%) and randomly distributed false-negative events. For each strain, we identified ~1400 events in one sequencing reaction, with a high resolution of recombination breakpoints (within 1kb). Consistent with our hypothesis, we found a negative association between the enrichment of H3K9me3 and crossover rates in the euchromatic regions in both strains, and we are further quantifying the contribution of TEs to such association. Furthermore, our orthogonal approach that counts the number of recombinants between two visible markers also supports a reduced recombination rate caused by the presence of TEs. Overall, with our newly developed approaches, our investigations reveal how TEs influence recombination rates by altering the chromatin landscapes.

537F **BRWD3 targets KDM5/Lid for degradation to maintain H3K4 methylation levels** Dongsheng Han[1], Samantha Schaffner[1,1], Jonathan P Davies[1], Lars Plate[2], Jared T Nordman[11]Department of Biological Sciences, [2]Department of Chemistry

Histone modifications are critical for regulating chromatin structure and gene expression. Dysregulation of histone modification levels may contribute to disease development and cancer. Therefore, understanding histone modifications is essential for development and disease. The chromatin-binding protein BRWD3, a known substrate-specificity factor of the Cul4-DDB1 E3 ubiquitin ligase complex, is required for maintaining active histone modification levels. Loss of BRWD3 function causes an increase in H3K4me1 levels. The underline mechanism, however, is unknown. We found that BRWD3 depletion also causes a decrease in H3K4me3 levels. To reveal the mechanism by which BRWD3 regulates the H3K4 methylation levels, we performed BRWD3-IP mass-spectrometry. We identified an interaction between BRWD3 and the lysine-specific demethylase 5 (KDM5/Lid), an enzyme that removes tri- and di- methyl marks from lysine 4 on histone H3. Moreover, analysis of ChIP-seq data revealed that BRWD3 and KDM5 are significantly co-localized throughout the genome. We show that BRWD3 promotes K48-linked ubiquitination of KDM5. Consistent with this, KDM5/Lid is rapidly degraded in a proteasome-dependent manner with a half-life of less than 30 mins. Critically, KDM5/Lid degradation is dependent on both BRWD3 and Cul4. In addition, we have found that *BRWD3* is suppressor of Position-effect variegation (PEV). Loss of a single copy of *KDM5*, however, partially rescues the the *BRWD3* PEV phenotype. Our results suggest that BRWD3 targets KDM5/Lid for degradation to ensure the balance of H3K4me levels.

538F **Sequence Divergence and Binding Factors of Repetitive Histone Loci** Connor Smith[1], Lauren Hodkinson[2], Leila Rieder[31]Emory, [2]Graduate Division of Biological and Biomedical Sciences, Emory Genetics and Molecular Biology, [3]Biology, Emory

The regulation of replication-dependent histone genes is required for genome stability and gene regulation. In many species, histone genes are clustered, allowing unified regulation. The regulation is accomplished by the assemblies surrounding the histone gene clusters, collectively known as the histone locus body (HLB). The histone locus body contains transcription and processing factors important for replication-dependent histone expression. Currently, knowledge of conserved histone regulatory mechanisms is limited in *Drosophila*; sequencing and annotation of repetitive histone loci is challenging and we do not know all the factors that target the histone loci and contribute to regulation. From the few species' loci that have been annotated, we note differences in arrangement, sequence similarity, chromosomal location, and number of loci. This indicates that locus heterogeneity might point us toward conserved regulatory mechanisms.

To expand knowledge of HLB associated factors and divergence of sequences, we are conducting two studies to elucidate histone gene organization and regulation in *Drosophila*. We are aligning publicly available ChIP-seq datasets to the *D. melanogaster* histone gene array. Through this screening, we identified novel DNA-binding factors that may be present in

the histone locus body. We previously performed this screen via a candidate-based approach and are now extending to an unbiased collection of DNA-binding factors. To expand understanding of histone locus organization, we are annotating the histone loci from diverse species using recently available genome assemblies from long read sequencing. Combined, these studies will provide us with a better picture of histone gene organization, and the regulatory components that localize to histone genes.

539F **Analysis of repetitive DNA elucidates details about the composition of the *D. melanogaster* B chromosomes** Ana Beatriz Stein Machado Ferretti[1], Diogo Milani[1], Emiliano Martí[2], Maria Dulcetti Vibranovski[3,4], Stacey L Hanlon[5], Diogo Cavalcanti Cabral de Mello[1,61]Departamento de Biologia Geral e Aplicada, Instituto de Biociências, Universidade Estadual Paulista (UNESP), [2]Department of Biology, University of Rochester, [3]School of Mathematical and Natural Sciences, New College, Arizona State University, [4]Depto. de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, [5]Department of Molecular and Cell Biology & Institute for Systems Genomics, University of Connecticut, [6]Department of Experimental Biology, Genetics Area, Universidad of Jaén

The essential set of chromosomes for an individual form the A complement, which includes the autosomes and sex chromosomes. However, in several species the presence of extra chromosomes, or B chromosomes, has been documented. These chromosomes are not an essential part of the genome, but it has been shown that their presence can cause changes in cell metabolism, altering the gene expression. Recently, B chromosomes were found in *Drosophila melanogaster*, a model organism with an abundance of resources and information that allows for a deep investigation into B chromosome biology. The B chromosomes are mostly heterochromatic and are likely an isochromosome that arose from Chromosome 4 since both share the *AAGAT* satellite repeat. In this work we provide more details about the composition, origin and evolution of B chromosomes of *D. melanogaster*. We analyzed the sequences of six genomes that either carried B chromosomes (+B) or did not (0B) and identified repetitive DNA through RepeatExplorer, followed by the Repbase library to recover previously described families, and analyzed the families in quantity and divergence through RepeatMasker. In order to understand sequence sharing among B chromosomes and complement A, the prospected repetitive DNAs were mapped by fluorescent *in situ* hybridization (FISH) on mitotic *D. melanogaster* chromosomes. Overall, there were analyzed 183 families of TEs, 25 types of microsatellites and ten of satDNA sequences. From the comparison between the 0B and +B samples, we found that several repetitive sequences were more prevalent in the +B genomes and were five satellite DNA (satDNA) families, 16 microsatellites, and 40 transposable elements (TEs). SatDNA analyses revealed six previously unknown families for *D. melanogaster*'s genome, and FISH analyses showed signals for two of those families, which were clustered on B chromosomes and along all centromeric regions of the autosomes and X chromosome. Similarly, the microsatellites that are carried on the B chromosomes also exhibit signals on other chromosomes in addition of Chromosome 4. These data indicated that B chromosomes in *D. melanogaster* may have originated from Chromosome 4, however, these chromosomes accumulated sequences present in other chromosomes throughout their differentiation, indicating that the B chromosome is a mosaic of distinct elements.

540F **Regulation of gene expression by HP1 proteins** Annesha King, John Schoelz, Nicole RiddleUniversity of Alabama at Birmingham

Heterochromatin Protein 1a (HP1a) was discovered in Drosophila and is a major component of heterochromatin. HP1 proteins contribute both to genome stability and gene regulation. In *Drosophila melanogaster,* there are three somatically expressed HP1 proteins: HP1a, HP1b, and HP1c. HP1a is an essential protein and necessary for genome integrity due to its functions at the centromere and telomere. While enriched in heterochromatin, it has binding targets in heterochromatin and euchromatin, and it acts as both a repressor and activator of gene expression. HP1b and HP1c also have binding targets in heterochromatin and euchromatin, but most of their binding sites are in euchromatin. Thus, all three proteins are involved in the regulation of gene expression, and analysis of ChIP-seq data suggests that they share many binding sites. To understand the contribution of the HP1 proteins to gene regulation, elastic net regression models were utilized to model the impact of HP1 binding on gene expression. These models suggest that HP1b is of particular importance, but that genomic features including promoter motifs, accessibility, and sequence composition also contribute. In this study, we test the model predictions by measuring gene expression changes after recruitment of HP1 proteins to endogenous genes a CRISPR/dCas9 system. When HP1b and HP1c were targeted to promoters, we observed significant activation at 50% of sites, while with HP1a, we observed repression at one of 6 sites. These results suggest that HP1b and HP1c typically act as activators, but that genomic context is important in determining expression outcomes at specific sites.

541F **Investigating the role of Polycomb repression in *Drosophila* eye specification** Haley E. Brown, Justin

KumarBiology, Indiana University

During metazoan development, gene regulatory networks (GRNs) are activated in undifferentiated tissues to induce a specific fate. However, when GRNs are disrupted, the tissue can *transdetermine* – losing the programmed fate to adopt another. Epigenetic factors, such as the Polycomb Group (PcG) proteins, ensure proper spatiotemporal control of GRNs. PcG proteins function as a set of complexes to add a repressive histone mark (H3K27me3) and condense chromatin. In turn, the accessibility of chromatin – or lack thereof – regulates differential transcription of genes in certain tissues. While the correlation between GRNs and chromatin modifications in development is widely established, the underlying mechanisms linking the two during transdetermination has yet to be discovered. *The overarching goal of this project is to determine how epigenetic modifications affect tissue fate specification.* An excellent model to study the mechanisms underlying fate plasticity is the eye-to-wing transformation of *Drosophila* eye-antennal discs (EADs). Previous work from our lab discovered that the EAD-specific removal of one PcG protein, Polycomb (Pc), transforms the eye imaginal tissue to wing – indicating that the loss of epigenetic repression is sufficient to allow cellular reprogramming.

To investigate the molecular mechanism underlying this transformation, I have performed RNA-seq on wild-type (WT) wing discs (WDs) as well as WT and *Pc* knockdown EADs throughout third instar development. This analysis identified 13 candidate genes that could be responsible for promoting reprogramming of the EAD. My preliminary data suggest the most promising of these candidates is *vestigial* (*vg*), as this locus is directly regulated by Pc. CUT&RUN analysis likewise shows the epigenome profile of candidates in *Pc* knockdown EADs transition to favor that of WDs. Furthermore, overexpression of *vg* in the EAD grants an eye-to-wing transformation, and ectopic *vg* expression is detected in the developing wing pouch of the transformed disc. To determine whether Vg is sufficient for the *Pc*-dependent eye-to-wing transformation, we knocked down *Pc* in vg^{1} or sd^{1} hypomporphic mutant backgrounds. Interestingly, when *Pc* is knocked down in either background, the direct eye-to-wing fate is lost and instead the disc hyperproliferates and adopts the fate of other imaginal discs as well as non-imaginal tissue. Here, we propose that Vg is the master regulator directing wing fate. Once this primary push towards wing fate is gone, other selector genes attempt to take control of the tissue and contribute to the specification of multiple new fates.

542F **3D dynamics of enhancer-promoter interactions during transvection in living *Drosophila* embryos** Hao Deng, Bomyi LimChemical and Biomolecular Engineering, University of Pennsylvania

Many previous studies demonstrated evidence that an enhancer and promoter need to be in close proximity to recruit RNA Polymerase II (Pol II) and initiate transcription. Recent efforts to measure the distance between active enhancers and their target promoters suggest that it is approximately 200~400 nm. However, most of the previous works have been done with fixed cells that provide limited temporal information, and hence the kinetics of how enhancers interact with the target promoters are still poorly understood. We study the dynamics of enhancer-promoter communications during transvection in early *Drosophila* embryos. In our transvection assay, the enhancer and its promoter are placed at the homologous positions of two different alleles. The alleles would only be in proximity of one another during active transcription in *trans* with the help of insulators. The drastic change in distance allows easy distinction between non-paired and paired states of the enhancer-promoter, making the transvection assay a favorable platform to study the dynamics of the interactions. With the combination of ParS/ParB-mediated DNA labeling and MS2/MCP-mediated RNA labeling, we measured the distance between an enhancer and its target promoter across homologous chromosomes in transcriptionally active and inactive nuclei. We found that the enhancer and the target promoter have to sustain a close distance for an extended time to transcribe *in trans*. However, the close proximity itself does not guarantee immediate trans-activation, as some nuclei showed transcription after some delay despite close allelic association. Our quantification also revealed no obvious correlation between the enhancer-promoter proximity and transcriptional bursting. In sum, our study provides better temporal understanding on the mechanism of enhancer-mediated transcriptional activation.

543F **Triggering targeted recombination with hybrid transposable elements** Victoria Lopez[1,2,3], Stephanie Mauthner[1,2], W. Daniel Tracey[1,2,31]Gill Center for Biomolecular Science, Indiana University, [2]Department of Biology, Indiana University, [3]Program in Neuroscience, Indiana University

P-elements, piggybac transposons, and Minos transposons, are different types of transposons that are commonly used in *Drosophila* genetics. Transposons are flanked by terminal inverted repeat (TIR) sequences at both ends and these sequences vary by transposon type. Many fly strains with molecularly defined deficiencies have been generated from FLP-FRT mediated recombination between FRT-bearing transposons, which often results in the generation of

hybrid transposable elements. In hybrid transposable elements, or hybrid elements, the TIR sequences are derived from distinct transposable elements which renders them immobile and incapable of further transposition. We were interested in determining whether flies containing hybrid elements can be used to trigger recombination in male flies as has been described for intact mobile transposable elements. While recombination does not normally occur in the male germline of *Drosophila*, prior studies showed that male recombination can occur when p-elements are mobilized by a transposase source. To test our hypothesis, we used a hybrid element deficiency of the *smoke alarm* (*smal*) gene, which is important for normal thermal nociception behavior. *smal*-deficient (*smal*-df) mutants made through a FLP-FRT-based deletion contain a hybrid element derived from a p-element and a piggybac transposable element. Our goal is to generate a recombinant with a closely linked mutation on the second chromosome. We generated flies that contained the *smal*-df, p-transposase, and visible markers which allowed us to identify recombinant progeny. We screened 3,901 progeny of males containing the hybrid element in the presence of a p-transposase source and identified 7 recombinant progeny. This recombination frequency of 0.00179 is significantly higher than meiotic recombination in male *Drosophila melanagaster*. Thus, our findings suggest that p-transposase can induce male recombination in flies containing hybrid elements. This allows for targeted recombination at defined genetic loci in the absence of p-element hopping.

544F **A *Drosophila* Model for Trinucleotide Repeat-Induced Silencing in Friedrech's Ataxia melanogaster** Nhi NT Vuong, Andrew M ArshamBemidji State University

Friedrech's Ataxia (FRDA) is the most common inherited ataxia, an autosomal recessive condition characterized by progressive ataxia, dysarthria, weakness, fatigue, and cardiomyopathy. The progression of FRDA results in considerable morbidity and premature mortality within 30 to 40 years. The key factor causing FRDA is silencing of the nuclear gene encoding the mitochondrial protein frataxin, caused by expansion of GAA trinucleotide repeat tracts. The frataxin protein is required for ATP production and iron detoxification (Rodden et al., 2022). In non-FRDA cells, the first intron of *FXN*, which typically consists of less than 30 triplets of GAA_n, is capable of synthesizing functional frataxin. In FRDA cells, GAA_n ranges from 100 to 1500 triplets, resulting in prematurely terminated transcripts (mRNA-ptt) upstream of the repeats, leading to the formation of a novel, truncated, and stable mRNA. These mRNA-ptt decrease the synthesis of functional frataxin proteins. Studies suggest that the magnitude of GAA_n expansion correlates with increased aberrant transcripts, and also indicated hyper-methylation develops within the vicinity of the GAA-repeat expansion, subjecting the *FXN* gene to heterochromatin silencing (Yanjie Li et al., 2022, Finsterer 2022). Despite FRDA having been widely studied in mammalian models, there is no direct evidence to demonstrate the mechanism of heterochromatin formation. Here we report a *Drosophila melanogaster* model for trinucleotide repeat-induced silencing that contains a P-element with a white+ reporter gene adjacent to 310 tandemly arrayed GAA repeats. We carried out a transposition mutagenesis screen to better understand cis- and trans-acting factors causing triplet expansion-associated silencing. Reporter gene expression was variegated in a small number of insertional mutants, but the vast majority of mutants had full reporter expression, indicating that the GAA array itself can trigger silencing under some conditions but does not trigger silencing more broadly. Our results suggest that additional local or topological factors are required for silencing.

545F **Evolutionary origins and diversification of variant histone H2Av in *Drosophila*** Pravrutha Raman[1], Sierra Simmerman[1,2], Ashlyn Anderson[2,3], Toshio Tsukiyama[1], Harmit S Malik[1,21]Basic Sciences, Fred Hutchinson Cancer Center, [2]Howard Hughes Medical Institute, [3]Basic Sciences, Fred Hutch Cancer Center

Histones and their variants package DNA and facilitate crucial biological processes in eukaryotes. Core histones primarily package DNA while histone variants can replace core histones to enable specialized functions, such as DNA repair, epigenetic inheritance, or chromosome segregation. Most eukaryotes (including humans) encode core histone H2A and two universal variants, H2A.Z and H2A.X, which function in gene regulation and genome repair, respectively.

Unexpectedly, H2A.Z and H2A.X are fused into a single chimeric histone gene, *H2Av*, in *Drosophila* species. We traced the evolutionary origins of this unusual histone variant using phylogenomic analyses of insect species. We found that early-branching Diptera such as mosquitoes have H2A repertoires resembling that of other eukaryotes with independent H2A.X and H2A.Z histones. However, *H2Av* was born within Diptera as a monophyletic event, via the addition of a SQ motif containing C-terminal extension from H2A.X onto the existing *H2A.Z* gene in the same shared syntenic location.

Our analyses also unexpectedly revealed two independent duplications of H2Av within *Drosophila* in the oriental (*takahashii/ suzukii*) and *ananassae* subgroups. The ancestral *H2Av* gene is still retained across these species. We name the new duplicates *H2Av2* (oriental subgroup) and *H2Av3* (*ananassae* subgroup). H2Av2 and H2Av3 have a relatively conserved histone fold domain but show a dramatic divergence in their N- and C- terminal tail sequences. Notably, the

C-terminal SQ motif that is essential for the DNA damage repair function of H2Av (and H2A.X) is maintained in H2Av2 but not in H2Av3. Lastly, while the ancestral histone H2Av is expressed ubiquitously, both new duplicates show enriched expression in males compared to females. Together, these results suggest *H2Av2* and *H2Av3* have acquired new histone functions in males, with the DNA damage repair function retained in H2Av2 but not H2Av3. We are characterizing these H2Av duplications in native species and in *D. melanogaster* to determine how histone innovation can contribute to genome regulation, integrity, or potentially novel, germline-specific functions.

546F **Describing genetic interactions between blm and rDNA in Drosophila** Keith A Maggert[1], Ergul Susamci[21]Molecular and Cellular Biology, The University of Arizona, [2]Genetics GIDP, The University of Arizona

The BLM DNA helicase is a member of the RecQ family of ATP-dependent helicases. The *Drosophila* ortholog, *blm*, is encoded by the *mus309* locus. Absence in humans gives rise to Bloom›s Syndrome, a rare, autosomal recessive disorder characterized by proportional dwarfism, sterility, and predisposition to many different cancers. Analysis of Bloom Syndrome clinical entities and patient-derived cell lines have shown a defect preferentially in some hard-to-replicate DNAs, including those that are packaged as heterochromatin, that are repetitive, that form R-loops or quadraplexes, or are highly-expressed. The large (*35S*) primary ribosomal RNA gene arrays (*rDNA*) exhibit all of these characteristics, which motivated us to look for genetic interactions between *blm* and the *rDNA*.

The *35S rDNA* loci (*Xbb* and *Ybb*) are composed of hundreds of tandemly duplicated *rRNA* genes – the *18S*,*5.8S*, and *28S* subunits. They are among the most unstable genetic elements due to their repetitive nature. In our study, we addressed outstanding questions regarding the consequence of *blm* defects on the rDNA using *Drosophila* and human patient-derived cell lines. Using classical and new CRISPR-mediated deletions, we found a strong genetic interaction between *blm* and the *rDNA*. *blm* enhanced the bobbed *rDNA* deficiency phenotype, mostly through a persistent *blm*-mediated loss of *rDNA* copy number. This reduction was attended by cytological defects in nucleolar structure. *blm* mutants produced a high frequency of *X-Y* exchanges at the *rDNA*. The translocations and *rDNA* copy number losses were fixed in individual lineages after formation, showing that *blm* has transgenerational effects that cannot be easily repaired. *blm* mutants also produced rare magnified *rDNA* arrays. Our data suggest that *blm* stabilizes *rDNA*, and defects lead to hypervariability – usually appearing as loss, but occasionally appearing as gains – in *rDNA* copy number.

Key portions of this hypothesis were recapitulated in human cells. We determined *rDNA* copy numbers by qPCR in 3 different human cell lines as well as in blood samples collected from Bloom Syndrome patients; mutations in the BLM gene cause hypervariability in the *rDNA* copy number, as in *Drosophila*.

547S **Observing the Interchromosomal Effect in *D. simulans*** Nigel Muhammad-Lahbabi, Nicole CrownCase Western Reserve University

The interchromosomal (IC) effect is the genome wide increase in crossover (CO) events and decrease in non-crossover (NCO) events in response to the presence of a heterozygous inversion. Interestingly, there is no change in double strand breaks (DSB) formed compared to wildtype. These findings suggest plasticity in the decision for how to repair DSBs during recombination. To further understand sources of this plasticity, we will use a comparative biological approach and analyze the interchromosomal effect in the closely related sister species, *D. simulans*. Until recently, only one inversion had been identified in *D. simulans,* suggesting that this species does not normally form inversions. However, recently the Stern lab created an inversion on chromosome 3R, allowing us to test whether there is an interchromosomal effect in *D. simulans.* To do this, we are setting up genetic crosses using this inversion to determine the CO frequency on other non-inverted chromosomes. In order to further shed light on the IC effect in *D. simulans*, we are cytologically characterizing the synaptonemal complex and DSB formation during prophase. Together, these experiments will help us determine if the IC effect is present in *D. simulans.*

548S **Heterochromatinization of Repetitive DNA is Location Dependent** “Alix” Brittny Hathaway, Safiyo Aden, Andrew ArshamBiology, Bemidji State University

Heterochromatin, though gene-poor, is nonetheless essential for cellular function and organismal survival and plays important roles in gene expression, genomic stability, and defense against invasive DNA. How a cell’s epigenetic machinery recognizes and neutralizes novel threats, such as repetitive DNA, remains unclear. Here we show that

the recognition and silencing of an exogenous tandem array of repetitive DNA is highly location-dependent. Using transposition mutagenesis, we inserted a reporter construct expressing the white gene adjacent to a 256-copy tandem array of the 36 nucleotide E. coli lac operator. Only ~1% of recovered flies expressed variegated eye color, indicating that the repeat array was not itself sufficient to trigger silencing. Many of the variegating insertions are located in gene-rich euchromatic regions, often in promoters or 5' UTRs, showing that under the right conditions the lacO array can trigger the formation of ectopic heterochromatin even in actively transcribed euchromatin. 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.

549S **GAGA-Associated Factor Fosters Loop Formation in the *Drosophila* Genome** Xiao Li[1], Xiaona Tang[2], Xinyang Bing[1,3], Christopher Catalano[1], Taibo Li[2], Gabriel Dolsten[1], Carl Wu[2], Michael Levine[11]Princeton University, [2]Johns Hopkins University Schools of Medicine, [3]BlueRock Therapeutics

The role of genome organization in the control of gene expression persists as a central problem of regulatory biology. Most efforts have focused on the role of boundary elements and CTCF in the compartmentalization of the genome into a series of topological associating domains (TADs). These compartments have been suggested to enable long-range DNA-DNA associations via loop extrusion processes. However, there is emerging evidence for long-range focal contacts, whereby specific DNA sequences associated with promoters and distal enhancers interact to form chromatin loops. One such class of DNAs, tethering elements, binds GAGA-associated factor (GAF). Previous studies provided evidence that GAF possesses amyloid properties *in vitro*, enabling the formation of loops bridging separate DNA molecules. In this study we investigated the possibility that GAF also functions as a looping factor in *Drosophila* development. We employed a combination of Micro-C assays, proteasomal degradation, and genome editing to examine the impact of defined GAF mutants on genome topology. These studies suggest that the N-terminal POZ/BTB oligomerization domain is particularly important for long-range interactions of GAF bound to distant GAGA-rich tethering elements. By contrast, the C-terminal low complexity domain (poly Q) plays only a minor role in loop formation. The most striking effects of GAF mutants are observed for long-range promoter-promoter interactions that coordinate the activities of distant paralogous genes.

550S **Using wavelets to generate new insights into the impact of biological processes on the 3D nuclear architecture of *Drosophila*** Ryan Pellow, Josep ComeronDepartment of Biology, University of Iowa

Many cellular phenotypes implicated in development and disease are associated with molecular attributes such as gene expression but also with variations in the 3D nuclear organization of genomes. Genomic advances have revealed multiscale structures including loops, topologically associating domains (TADs) and chromosome territories that impinge on the molecular activity of genes and ultimately cells. Different methodologies to characterize loops and TADs, however, often reveal different structures with varying levels of accuracy and sensitivity. Currently, the most advanced methods to infer 3D nuclear organization produce different outcomes depending on the resolution of the analysis, generate results that are purely qualitative (presence/absence of loops or TADs), and do not directly infer overlapping or hierarchical structures that can exist in a collection of cells. To address these challenges, we have developed WaveTAD, a wavelet transform-based method that describes the 3D nuclear organization in a probabilistic, resolution-free, and hierarchical manner. High-resolution imaging confirmed the compartment-sized TADs called by this new method. WaveTAD also demonstrated an ability to call TADs in heterogeneous samples and with low signal-to noise ratio. We also show that the probabilities generated by WaveTAD capture variable frequency of structures across genomes and between samples. Applying this method to embryonic cells, the 3D nuclear hierarchy is recapitulated in early-stage fly embryos suggesting that the hierarchical TAD structure is present early in development in a subset of nuclei. After applying clustering techniques to identify the synergistic interactions between architectural proteins, accessory proteins, and histone marks, we show that different biological processes (e.g., dosage compensation, transcription rates, heat-shock response, crossover rates, presence of R-loops, etc.) are associated with different TAD structures and TAD strengths.

551S **Its *abo*ut Time: Abnormal oocyte's (abo) role in embryonic histone gene regulation** Eric Albanese[1], Casey E Schmidt[2], Leila E Rieder[21]Emory University, [2]Biology, Emory University

The early *Drosophila melanogaster* embryo must maintain a careful balance between gene activation, repression, and DNA replication. The histone genes sit in the middle of this process: a surplus or deficit of histones leads to dire consequences. One regulator of histone expression is *abnormal oocyte* (*abo*), a *Drosophila* maternal effect gene first

characterized by Larry Sandler in 1970. Loss-of-function mutations in *abo* cause defects in embryonic development. Later characterization of *abo* revealed that it negatively regulates histone gene expression—likely through targeting *histone* promoters. Despite these important experiments, *abo* has received little attention since the early 2000s, and the mechanism by which it contributes to histone repression is unknown. Thus, our goal is to define the molecular role of *abo* in histone gene expression. To that end, we generated V5-tagged *abo* lines via CRISPR. Using these lines, we determined that Abo targets the endogenous histone gene cluster in blastoderm-stage and later embryos. We will now assess the interplay of Abo with other factors that negatively regulate histone genes, such as muscle wasted. We will determine if reducing histone gene copy number results in ablation of Abo localization, as is the case for mute. We will also define the spatiotemporal localization patterns for Abo, as it may only be present at the histone gene array during specific cell cycle stages or developmental times. Furthermore, we will test if these negative regulatory factors can functionally compensate for each other by using available mutant and rescue transgene lines. Histone transcription is a highly complicated system, so understanding the relationships between negative regulators and their target genes can create better models for highly regulated genes.

552S **The role and control of gene expression variation underlying tissue-specific responses to copper stress in *Drosophila melanogaster*** Elizabeth Everman, Stuart MacdonaldUniversity of Kansas

Heavy metal pollution represents a damaging and ubiquitous source of environmental stress that negatively impacts human and ecosystem health. Copper is one of a handful of biologically necessary heavy metals that is also a common pollutant. Under normal conditions, copper ions contribute to enzyme function, cellular respiration, and oxidative stress response. In excess, copper quickly results in cell damage and has been linked to neurological disorders and diseases with effects ranging from learning disability in children to exacerbation of Alzheimer's and Parkinson's Diseases. We used copper as a model for understanding the genetic basis of heavy metal resistance for its biological relevance and because many of the genes that respond to copper stress also interact with other more toxic metals such as lead, cadmium, and mercury. To identify genetic variants that influence the gene expression response to copper stress in a tissue-specific manner, we characterized the copper stress response in head and gut tissue of 96 inbred strains from the *Drosophila* Synthetic Population Resource (DSPR) using a combination of differential expression analysis and expression quantitative trait locus (eQTL) mapping. Differential expression analysis revealed clear distinctions in gene expression between head and gut tissue and due to interactions between tissue and treatment (control versus copper exposed flies). Examination of tissue- and treatment-specific expression patterns provided additional insight into previously reported expression responses to copper stress. eQTL mapping of gene expression under control and copper conditions as well as for the change in gene expression as a result of copper exposure (copper response eQTL) revealed hundreds of genes with tissue-specific local *cis*-eQTL and many distant *trans*-eQTL. Several eQTL had genotype by environment effects on gene expression under copper stress and were associated with genes known to play an important role in response to copper stress and toxins including one of the metallothionein genes *MtnA*, *Mdr49* and *Mdr50*, *Sod3*, and several members of the glutathione s transferase and cytochrome p450 gene families. Together, our data build a nuanced description of the roles and interactions between allelic and expression variation in copper-responsive genes, providing valuable insight into the genomic architecture of susceptibility to metal toxicity and highlighting many candidate genes for future functional validation.

553S ***Drosophila* Set8 has functions in cell proliferation and neurogenesis that are independent of its chromatin modifying activity** Aaron Crain, Megan Butler, Daniel J McKay, A. Gregory Matera, Robert J DuronioUNC Chapel Hill

Histone post-translational modifications (PTMs) regulate processes necessary for cell proliferation and gene expression by modulating DNA accessibility and the recruitment of trans-acting factors. Therefore, enzymes that deposit histone PTMs (writers) are crucial regulators of cell proliferation and gene expression. Set8 is a writer responsible for mono-methylating lysine 20 of histone H4 (H4K20me1). H4K20me1 is involved in numerous processes related to cell proliferation and gene expression, though its function has largely been indirectly interpreted by manipulating the function of Set8. However, this type of analysis can obfuscate the function of H4K20me1 because Set8 has non-histone substrates (i.e. p53 and PCNA) and non-catalytic functions (i.e. the DNA damage response). Thus, the field suffers from a significant gap in understanding which biological functions of Set8 are attributable to H4K20me1 and which to non-histone substrates or non-catalytic interactions. Recent work in our lab has shown that *Drosophila* mutants expressing unmodifiable $H4^{K20A}$ or $H4^{K20R}$ are phenotypically distinct from $Set8^{null}$ mutants. We demonstrate in the eye that $H4^{K20A}$ and $H4^{K20R}$ mutant cells proliferate similarly to wild-type cells, whereas $Set8^{null}$ mutant cells cannot proliferate and die. Furthermore, third instar larvae expressing unmodifiable H4K20 have a distinct gene expression profile compared to $Set8^{null}$ larvae. Specifically, members of the Notch signaling pathway, genes involved in neurogenesis, and master

transcriptional regulators (including *zelda* and *engrailed)* are down-regulated in *Set8^{null}* suggesting critical functions of Set8 in a broad range of essential developmental pathways. Remarkably, most of the down-regulated genes in *Set8^{null}* are unchanged in *H4^{K20A}* larvae and only modestly down-regulated or unchanged in *H4^{K20R}* larvae. Taken together these data suggest that the developmental and cell proliferation defects observed in Set8^{null} animals are largely due to H4K20me1-independent effects on the expression of genes involved in essential developmental pathways. Given that perturbations of Set8 have been implicated in several cancers, these findings and further investigation of Set8's function in essential developmental pathways could lead to the discovery of novel therapeutic targets, especially those that modulate cell proliferation.

554S **Investigating the *in vivo* functions of histone monoaminylation using *Drosophila*** Harim Delgado-Seo[1,2], Jung-Wan Mok[3], Gary Huang[4], Herman A. Dierick[4], Ian Maze[5], Shinya Yamamoto[1,4,61]Department of Neuroscience, Baylor College of Medicine, [2]Department of Neuroscience, Jan and Dan Duncan Neurological Research Institute, [3]Department of Molecular and Human Genetics, Jan and Dan Duncan Neurological Research Institute, [4]Department of Molecular and Human Genetics, Baylor College of Medicine, [5]Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, [6]Jan and Dan Duncan Neurological Research Institute

Despite advances in our understanding of the neural mechanisms that mediate drug substance abuse and addiction, little is known about the genetic underpinnings of maladaptive and compulsive drug use. It is thought that increased risk of relapse is a long-term behavioral adaption that stems from the stable changes in the brain reward circuitry induced by prolonged drug abuse. Recent studies in rats have found that covalent binding of dopamine to glutamine 5 (Q5) on the histone H3.3, a process called dopaminylation, plays a critical role in cocaine-induced transcriptional plasticity in the ventral tegmental area (VTA). Reducing the dopaminyl substrate in H3.3 by expressing H3.3 Q5A variant in a dominant-negative manner subsequently attenuated cocaine-seeking behavior during withdrawal. Further studies have identified another glutamine residue on H3.3 onto which dopamine or other biogenic amines such as serotonin can be covalently conjugated (monoaminylation). However, the functional significance of this newly identified site as well as overall physiological importance of histone monoaminylation is understudied.

To overcome the issue of the genetic and cellular complexity observed in mammals and to advance our understanding of the epigenetic mechanisms underlying drug addiction, we are generating transgenic *Drosophila melanogaster* strains that allow manipulation of monoaminyl marks on H3.3. We designed 13 different transgenic constructs that alter the sequence of the H3.3 tail and are in the process of generating transgenic lines using phiC31 mediated targeted integration. Once established, we plan to cross each line individually to an array of ubiquitous or tissue/cell-type specific GAL4 driver lines (e.g. elav-GAL4, repo-GAL4, ple-GAL4) to test if overexpression of these mutant H3.3 causes any adverse phenotypes, primarily focusing on behavioral phenotypes such as basal locomotion, climbing, bang-sensitivity, courtship, aggression and physiological responses to cocaine. The outcome of this study will reveal if abolishing monaminylation marks on H3.3 has physiological consequences in *Drosophila*. Furthermore, identification of specific phenotypes caused by histone monaminylation defects will provide us with an entry point to further determine the precise function of this novel epigenetic mark *in vivo*.

555S **Super-resolution imaging of homologous chromosomes reveals different scales of genome organization in *Drosophila*** Jumana AlHaj Abed[1], Irene Farabella[2], Antonios Lioutas[1], Sarah Aufmkolk[1], Guy Nir[3], Chao.Ting Wu[11]Harvard Medical School, [2]Center for Human Technologies, [3]University of Texas Medical Branch

Somatic homolog pairing events are rare and transient in mammals, albeit critical and associated with processes such as DNA repair. In contrast, *Drosophila* homologs are paired in interphase somatic cells, and pairing-dependent gene regulation is detected at many loci, making it ideal for studying *trans* chromosomal interactions. Our recent work using haplotype-resolved Hi-C have shown that homologs pair relatively precisely, albeit variably, while establishing *trans*-homolog domains and loops at the population scale. We were motivated by these results, and especially the variation of pairing precision, to understand the implications for these findings at the single-cell level. For example, what is the relationship between pairing precision at specific loci with gene expression or epigenetic state? Here, using OligoSTORM we image homologs at super-resolution to reveal the structure of pairing, including Hi-C defined pairing precision and distances between homologs at different genomic scales ranging from megabases to tens of kilobases. First, sub-megabase chromosome walks along three different homologous chromosomal regions on 2L, 2R and 3L reveal differences in biophysical properties of paired chromosomes. In addition, using Homolog Specific Oligopaints (HOPs) which target single nucleotide differences between homologous chromosomes in a hybrid cell line, we can distinguish paternal and maternal chromosomes and determine the extent of their physical proximity. Overall, we found evidence

for extensive intermingling between homologs within a 100 nm. Excitingly, chromosome walking with smaller, equal-size steps uncovers differences in the distances between homologs within different types of pairing. Finally, transcriptional inhibition perturbed pairing levels, revealing potential interplay between the structure of pairing and gene regulation. These findings elucidating the allele-specific principles of genome organization are a first step to addressing the *trans* chromosomal structure-function relationship in specialized tissue and in other species.

556S **Evidence for a trans-nuclear envelope bridge required for centromere tethering in *Drosophila melanogaster* neuroblasts** Jennifer A Taylor, Clemens CabernardBiology, University of Washington

Within the nucleus, chromatin is packaged in a precise and orderly fashion, which is vital to cellular and organismal functions including development, chromosome stability, and regulating transcription. Centromere positioning is a poorly understood component of genome organization, despite the fact that non-random centromere positioning occurs in a wide diversity of organisms and was first observed over 100 years ago. In *Drosophila melanogaster* larvae, the apical centrosome nucleates microtubules throughout the cell cycle in neuroblasts. Our lab recently reported that neuroblast centromeres are tethered at the apical periphery of the nucleus throughout interphase in a microtubule-dependent manner. Given that microtubules are generally separated from centromeres by the nuclear envelope, we reasoned that other proteins are likely required to function as a trans-nuclear envelope bridge. Here, we report on a directed RNAi screen using live fluorescence imaging of centromeres and microtubules in larval *D. melanogaster* brain explants. Knockdown of several nucleoporins as well as the cytoplasmic dynein heavy chain Dhc64C resulted in a number of neuroblasts in which centromeres became untethered even though the apical centrosomes retained microtubule nucleating activity. Based on these results, we propose a model in which cytoplasmic dynein links microtubules from the apical centrosome to nuclear pore complexes, which are in turn either directly or indirectly linked to centromeres. Future work will include additional mechanistic characterization and validation in addition to functional studies.

557S **Epigenetic silencing of transposons by nuclear and cytoplasmic factors of Piwi-piRNA pathway promotes Piwi's function in developmental robustness** Rasesh Y Parikh, Dhananjaya Nayak, Vamsi K GangarajuDepartment of Biochemistry and Molecular Biology and Hollings Cancer Center, Medical University of South Carolina

A significant portion of most animal genomes is occupied by repetitive sequences derived from transposable elements (TE), also known as 'jumping genes' that can jump from one genomic location to the other. Genome structural variants caused by TEs are implicated in sterility, various neurological disorders, and diseases like cancers, including ovarian cancer and leukemia; however, mechanisms that prevent the same remain largely unknown. TEs are primarily silenced at both transcriptional and post-transcriptional levels by piRNAs, a novel class of ~26 nucleotide-long germline-enriched small non-coding RNAs that bind the Piwi class of proteins. Our study focuses on the transcriptional silencing of TEs by the Piwi protein. Using fruit fly (*Drosophila melanogaster*) ovary as the model system, we introduce Modulo (Mod), a nuclear protein, and Nup358/RanBP2, a cytoplasmic component of the nuclear pore complex, as novel essential proteins for transcriptional silencing of TEs by Piwi protein. Transcriptional silencing of TEs involves four steps – 1) biogenesis of piRNAs that occurs in the cytoplasm, 2) binding of piRNAs to Piwi protein, 3) entry of Piwi-piRNA complex into the nucleus, and 4) epigenetic silencing of TE genomic sites. We show that Mod and Nup358 regulate piRNA function at distinct stages. Modulo is needed for the deposition of chromatin silencing epigenetic marks at transposon genomic sites. Modulo interacts with Panoramix, an essential protein in the transcriptional silencing of transposons, and regulates its ability to engage with target transposon RNAs, a critical step in the transcriptional silencing of transposons. Nup358, on the other hand, is needed for both piRNA biogenesis and entry of Piwi-piRNA complex into the nucleus. Nup358 anchors Piwi at the nuclear pore and couples piRNA biogenesis with the loading of piRNAs into Piwi- a prerequisite for Piwi entry into the nucleus. Thus, lack of Nup358 precludes Piwi from the nucleus and derails the transcriptional silencing of TEs. Taken together, our work introduces the function of two novel proteins in TE silencing. Furthermore, Mod and Nup358 mediated transposon silencing shows the functional aspect of Piwi's role in developmental robustness. In this context, we show that the lack of maternal role of Piwi predisposes somatic tissues of F1 to the activation of transposons that induce transcriptome instability with ensuing phenotypic variations. Both Mod and Nup358 are conserved in humans, and hence our work lays the foundation to explore the function of these proteins in human disease and in Piwi-piRNA pathway-mediated development robustness.

558S **Dissecting the temporal dynamics of histone inheritance through *Drosophila* neural development** Jason T Palladino, Xin ChenBiology, Johns Hopkins University

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 inheritance in *Drosophila* male germline stem cells (mGSCs). Further dissection of this process revealed it functions and is regulated in three-steps: 1) histone asymmetry is established during S-phase; 2) histone asymmetry is distinguished during M-phase; 3) the readout of the asymmetrically inherited histones guides asynchronous cell cycle progression following mitotic exit. 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. Preliminary data shows that 84% of dividing type-I NBs show asymmetry in total H3 segregation between renewed type-I NBs and their daughters, suggesting histone density asymmetry is conserved between mGSCs and NBs. However, the polarity of asymmetry is dynamic with all possible patterns being observed. As NB progeny identity is also dynamic and dictated by a transcription factor cascade, I hypothesize histone inheritance dynamics are associated with cell-fate determination in NB lineages.

Moving forward, I will 1) use live-cell imaging of staged larval brains to further characterize the temporal dynamics and patterns of histone inheritance in NBs, and 2) use single-cell multi-omics to elucidate epigenomic dynamics through development and identify candidate epigenomic and cell-fate regulators for neurogenesis. These works will both enhance our understanding of the roles of histone inheritance in ACD and elucidate the contributions and regulators of the epigenome during development.

559V **Insights into Magnification by a Functional Characterization of ribosomal DNA-null alleles in D. melanogaster** Selina Kindelay[1], Keith Maggert[21]Genetics GIDP, University of Arizona, [2]University of Arizona

The major loci for the large primary ribosomal RNA genes(35S rDNA, Xbb and Ybb) exist as hundreds to thousands of tandem repeats in all organisms, and dozens to hundreds in Drosophila. The rDNA accounts for over 60% of transcriptional output and 80% of steady-state cellular RNA. Despite the multiplicity, only a fraction (~50%) are actively transcribed at any one time, presumably through epigenetic means. A secondary regulatory system called nucleolar dominance selects Xbb or Ybb as the sole source of rRNA in males. Further, the unusual properties of the rDNA allow them to magnify, increasing their multiplicity, in particular unusual conditions. We recently proposed that rDNA magnification is a consequence of disrupted nucleolar dominance. The highly repetitive nature of the rDNA, its existence on multiple chromosomes, and its dynamic copy number variability make it difficult to investigate basic rDNA biology. Here we seek to gain further mechanistic insight into the epigenetic regulation, transcriptional activity, and overall development of mutants with altered or no active rDNA. Through targeted deletions in the rDNA of the Y chromosome, we have isolated and characterized rDNA null alleles and investigated the hypothetical “lower limit” of functional rDNA for organism viability. The remaining rDNA is mostly, if not all, inserted with R1 and R2 retrotransposons with R2 being highly expressed during the early 1st instar stages, identified as the lethal phase. These lethal lines, along with a previously generated and characterized semi-lethal allelic series of rDNA deletions has exhibited the presence of magnified progeny when crossed into various genetic backgrounds that destabilize heterochromatin-induced gene silencing (e.g., Su-(var)3-9), affect chromosome pairing, and when in the presence of specific X chromosomes. We have come across multiple mechanisms for the appearance of magnification, and we will present our most-recent work that unifies two longstanding observations in Drosophila: rDNA magnification and nucleolar dominance.

560V **Establishing the molecular mechanisms mediating chromatin architecture** Wenfan KeMolecular Biology, Princeton University

The eukaryotic genome is organized into looped domains in three-dimensional space, termed topologically associating domains (TADs). This organization is known to play a central role in gene regulation, development, and disease. TADs are delimited at each end by boundary elements (BEs). In addition to their architectural functions, BEs also have important genetic functions: they can block regulatory interactions between enhancers/silencers in one TAD and genes in another TAD; they can also mediate long-distance regulatory interactions. While the general features of chromosome organization have now come into view, little is known about how TADs are formed, or how specific long-distance

regulatory interactions take place. To investigate the mechanisms of TADs formation and BEs interaction, I focus on the well-characterized *Drosophila even-skipped(eve)* locus flanked by two BEs: *homie* and *nhomie*. Our previous studies have shown that *homie* and *nhomie* specifically pair with each other in an orientation-dependent manner. A dual reporter transgene system carrying *homie* or *nhomie* inserted -142kb upstream of *eve* locus has shown pairing interaction with endogenous *homie* and *nhomie*. Through chromatin conformation capture technique micro-C and transgene assays, I found that BEs pair with each other and exhibit distinct partner preferences and orientation dependence. Such properties define the looping topology of the TADs. This study shed lights on the fundamental mechanisms of TADs formation and chromatin architecture.

561V **Histone methylation regulates reproductive diapause in *Drosophila melanogaster*** Abigail DiVito Evans[1], Regina A Fairbanks[1,2], Paul Schmidt[1], Mia T Levine[11]Biology, University of Pennsylvania, [2]University of California, Davis

Fluctuating environments pose unique threats to developing organisms. To better match the immediate, local environment, many organisms adopt alternative developmental fates. The mechanism by which an individual, subjected to a suboptimal environment, exercises this “developmental plasticity” is poorly understood. Here we test the hypothesis that environment-dependent developmental plasticity is mediated by epigenetic factors. To model developmental plasticity, we exploit the reversible reproductive arrest of *Drosophila melanogaster* females called diapause. Using an inbred line, we demonstrate that diapause plasticity is epigenetic: only a subset of genetically identical individuals enter diapause, and this state of reproductive arrest is epigenetically transmitted for at least three generations. Furthermore, we show that active chromatin marks H3K4me3 and H3K36me1 are depleted in diapausing ovaries. Using ovary-specific RNAi against histone mark writers and erasers, we demonstrate that H3K4me3 and H3K36me1 depletion promotes diapause plasticity. Surprisingly, while H3K4me3 and H3K36me1 determine diapause plasticity in an inbred line with high plasticity, these two marks are similarly abundant across diapausing and reproductive ovaries in a genotypically distinct inbred line with low plasticity. This observation suggests that epigenetic regulation of reproductive diapause is variable between genotypes. Consistent with this possibility, the diapause-specific gene expression program also varies across the two genotypes. This study reveals new chromatin determinants of diapause plasticity and suggests that these determinants may be genotype-dependent. Our findings provide new insight into how organisms exploit epigenetic mechanisms to persist in fluctuating environments.

562V **Assessing an unprecedented role for Heterochromatin Protein 1a (HP1a) at mitochondria** Liliana Tullo[1], Francesca Cipressa[2], Giuseppe Bosso[3], Michela Di Salvio[4], Gianluca Cestra[4], Federico Caicci[5], Sofia Mauri[5], Elena Ziviani[5], Giovanni Cenci[61]Biology and Biotechnology “Charles Darwin”, University of Rome Sapienza, [2]Tuscia University, [3]Spanish National Cancer Centre (CNIO), [4]CNR/IBPM, [5]University of Padua, [6]University of Rome Sapienza

The Drosophila Heterochromatin Protein 1a (HP1a) is conserved non-histone protein with essential roles in heterochromatin maintenance and gene expression. We recently found that HP1a is also present on mitochondria, mostly associated with the outer mitochondrial membrane and with Drosophila VDAC1. Cellular fractionation from HeLa cells and from mouse liver and heart samples showed that also the mammal ortholog Hp1a localizes at mitochondria, further suggesting that HP1a is a mitochondrial resident protein and plays evolutionarily conserved roles in this organelle. Interestingly, loss of HP1a in Drosophila and in Hela cells associates with larger mitochondria number and increased mitochondrial mass without affecting mitochondrial biogenesis. This result prompted us to hypothesize a role for HP1a in the regulation of mitochondrial quality control and mitochondrial degradation. In support of this hypothesis, we recently found that a specific depletion of HP1a in Drosophila larval and adult muscles associates with impaired locomotor activity and altered mitochondrial ultrastructure, revealing an unexpected role of HP1a for mitochondrial activity at multiple levels during development

563V **Transcriptomic studies of histone H3 lysine 36 residue and writer enzyme mutants reveal unique and redundant functions** Harmony Salzler[1], Benjamin D McMichael[2], John C. Brown[3], Vasudha Vandadi[3], A.Gregory Matera[3,4,5,61]UNC Chapel Hill, [2]Quantitative Biology, UNC Chapel Hill, [3]IBGS, UNC Chapel Hill, [4]Biology, UNC Chapel Hill, [5]Genetics, UNC Chapel Hill, [6]Lineberger Comprehensive Cancer Center, UNC Chapel Hill

Histone post-translational modifications (PTMs) are critical regulators of the transcriptome. However, defining these regulatory roles unequivocally has been difficult due to the tendency of histone genes to exist in multi-copy arrays. Furthermore, mutation of enzymes that modify histones often produce phenotypes unrelated to histone PTMs due to their propensity to modify non-histone targets. To complement studies of histone modifying enzymes, we had previously developed a comprehensive histone gene replacement system to study histone tail residue mutations. In this work, we

utilize this system alongside H3 lysine methyltransferase (KMT) mutations to comprehensively investigate the roles of replication-dependent (H3.2) and replication-independent (H3.3) histone 3 lysine 36 (H3K36) on the transcriptome. Mono-, di-, tri-, and unmethylated states of H3K36 have been implicated in gene activation, gene repression, alternative splicing, dosage compensation, and M6A RNA modification in a variety of model systems.

Here, we present transcriptomic studies at multiple developmental timepoints featuring comparisons of writer and residue mutations. Our genomic analyses of H3.2K36R, H3.3K36R, and combined mutants have produced insights into the relative contribution and redundancy between H3.2K36 and H3.3K36 residues in these processes. Overall, our data suggest that H3.2K36 and H3.3K36 preferentially regulate different genomic compartments, but that each residue also exhibits the ability to compensate the reciprocal mutation to a large degree. Furthermore, comparison of H3K36R mutants with KMT mutants reveal a surprising degree of discordance in transcriptomic phenotypes with respect to processes that impact the abundance and structure of RNA. Overall, this study seeks to parse the relative contributions of H3K36 and KMTs in defining transcriptomic states originating in diverse genomic compartments.

564V **Creation of an optogenetically tagged Polycomb protein capable of inducible nuclear export in *Drosophila melanogaster*.** Sarah Aleman[1], Sean Johnsen[2], Daniel McKay[21]Biology, McDaniel College, [2]University of North Carolina Chapel Hill

Early in development there is a critical switch from transcription factor mediated to epigenetic mediated gene regulation. Polycomb Group proteins (PcGs) contribute to epigenetic gene regulation by heritably repressing transcription of target genes. Presently there exists two models to explain how Polycomb proteins initiate Polycomb domain formation at the earliest stages of embryogenesis: the instructive and responsive models. The instructive model posits that initiation of repression depends on PcG recruitment in a sequence specific manner, via direct interactions with DNA binding factors. By contrast, the responsive model proposes that the decision to initiate repression by PcG proteins is determined by the transcriptional state at target genes. The study of the proteins involved in this process, specifically PcGs, has been difficult as some of these proteins are essential within the first few hours after fertilization. To rectify this issue, we used the light-inducible nuclear export system, LEXY, optogenetic tag to create a fusion protein with Sfmbt, a subunit of the PcG Pho repressive complex (PhoRC). The LEXY tag will allow Sfmbt to be inducibly exported from the nucleus, thereby removing Sfmbt function. We used Gibson assembly to construct a plasmid encoding both the Sfmbt gene and the L-mCherry-LEXY cassette. We then transfected the assembled plasmid into Drosophila S2 cells. These cells were then visualized using fluorescence microscopy. This optogenetic approach, while promising, requires more research to optimize the conditions necessary to visualize inducible nuclear export. Once optimized this approach has potential for uncovering the function of PcGs in early development as well as further understanding when and how the switch from transcription factor regulation to epigenetic regulation occurs.

565T **Role of the yolk cell in *D. Melanogaster* midgut formation** Marina Sarantseva[1], Laurinas Pasakarnis[1], Damian Brunner[21]University of Zurich, [2]Molecular Life Sciences, University of Zurich

Around stage 13 of *D.melanogaster* development, two major morphogenetic rearrangements of embryogenesis take place in parallel: dorsal closure and midgut formation. While the process of dorsal closure is being extensively studied, the mechanism of midgut tissue spreading remains enigmatic. This project is focused on the last part of embryonic midgut formation – the dorsal ward spreading of midgut tissue along the yolk cell's surface that results in the formation of a continuous gut tube. Thereby, the yolk cell is fully engulfed by the midgut and subsequently degraded within the gut. Historically, researchers disregarded a possible contribution of the yolk cell to gut formation. However, our recent experiments suggest it plays an essential role. In particular, we detected non-muscle myosin-II waves deforming the yolk cell surface and found yolk cell-specific depletion of the non-muscle myosin-II light chain leading to midgut closure arrest. This work presents pioneering live imaging of actomyosin network dynamics at the yolk cell membrane and results obtained from the various yolk cell-specific genetic perturbations.

566T **Cellular consequences of acute manipulation of Rap1 activity in early embryonic morphogenesis** Amruta P Nayak[1], Michael A Glotzer[21]Cell and Molecular Biology Graduate Program, The University of Chicago, [2]Molecular Genetics and Cell Biology, The University of Chicago

The mechanisms with which embryos pattern force generation in space and time to shape specific organs and tissues are not well understood. Many large-scale tissue level morphogenetic events result from highly coordinated cell shape changes in epithelial cells which are driven by contractile forces generated from actomyosin cytoskeleton. While these epithelia undergo morphogenesis, they maintain integrity through mechanosensitive cell-cell adherens junctions that are

constantly remodeled to bring about the final tissue forms.

The small GTPase Rap1 is an attractive candidate for regulating the coordination and long-range force transmission throughout the tissue. Rap1 regulates the positioning of adherens junctions and their linkage to actin cytoskeleton. Rap1 has many functions complicating the analysis of its mechanistic role in a particular process. Therefore, to precisely dissect its role in dynamic morphogenetic processes, we have developed tools to monitor and optogenetically control Rap1 activity in Drosophila. We developed a Rap1 biosensor gives a direct readout for Rap1 activity. Our preliminary data indicate that Rap1 is highly active throughout the embryonic epithelium during embryogenesis. We have also developed tools to increase or decrease Rap1 activity in a spatiotemporally controlled manner using optogenetics. In particular, decreasing Rap1 activity using dominant negative (DN) Rap1 (optoRap1^{DN}) leads to a light-dependent decrease in embryonic viability. Further preliminary experiments indicate that this light-induced decrease in Rap1 activity also delays ventral furrowing, the first morphogenetic event in Drosophila gastrulation. This also reduces apical constriction and inhibits sealing of the ventral furrow. In future work, we will observe effect of Rap1 activity on adherens junctions and known Rap1 effectors and characterize how it influences the coordination of cell shape changes in dynamically deforming tissues.

567T **Real time dynamics of cell fate specification in the *Drosophila* tracheal system** Alison Simpkins[1], Robert Marmion[1], Stanislav Shvartsman[1,2,31]Lewis-Sigler Institute for Integrative Genomics, Princeton University, [2]Molecular Biology, Princeton University, [3]Flatiron Institute, Simons Foundation

Understanding the real time dynamics responsible for generating different cell fates is essential not only for understanding typical development but also for understanding how development is altered by mutations associated with developmental disorders, some of the most poorly understood human diseases. Current transcriptomics, proteomics, in situ hybridization and immunofluorescence techniques can provide information on expression states of cells. However, these technologies have fundamental limitations as they do not provide real time information on how the system is changing and face hard limits on the number of proteins and transcripts that can be visualized at one time. We have chosen the dorsal branches of the larval tracheal system of *D. melanogaster* as a model system for studying real time dynamics of cell fate specification. The dorsal branches, which begin developing during embryogenesis, represent a small primordium of migratory cells from which cells are selected to adopt specific fates. Typically, from this 5-8 cell primordium, one cell is selected to become an extensively branched terminal cell (TC) that supplies oxygen to target tissues, and another is selected to become a fusion cell (FC), fusing with other FCs in the network. FGF/ERK signaling is known to play a key role in the specification of the TCs and FCs of the dorsal branches. Additionally, the transcription factors *Drosophila serum response factor* (*DSRF*) and *escargot* (*esg*) have been identified as TC and FC-specific markers, respectively. To characterize the dynamics of cell fate specification in the *Drosophila* trachea with greatly improved temporal resolution, we used a combination of CRISPR-Cas9 genome editing, live imaging, and data integration. We used CRISPR-Cas9 genome editing to endogenously tag transcription factors and FGF/ERK signaling components including *DSRF*, *esg*, *sprouty* (*sty*), and *capicua* (*cic*) with fluorescent proteins and performed time-lapse imaging in *Drosophila* embryos. Following segmentation and tracking of dorsal branch cells, temporal, morphological, and expression information was extracted and integrated to obtain a complete characterization of the different cell types present as well as the dynamics of their protein expression. We have also applied this analysis to developmental disease-associated mutants; we show that these mutants often have ectopic terminal cells and characterize the expression profiles of these ectopic cells.

568T **Developmental Variation in the Rate of Collagen Deposition in the Cardiac Basement Membrane** Danielle MacDuff[1], Roger Jacobs[21]Biology, McMaster University, [2]McMaster University

Cardiovascular disease is a leading cause of morbidity worldwide. Many cardiomyopathies and developmental defects arise from misregulation of the cardiac extracellular matrix (ECM), a dynamic network of proteins, growth factors, and signaling molecules that acts as a protective sheath around organs and tissues. Changes in ECM composition are mediated in part by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). Dysregulation of the ECM leads to myriad outcomes, such as fibrotic scarring, hypertrophy, and myocardial infarction. Although fundamental to heart formation and function, the regulation of ECM integration and remodeling during growth is poorly understood. To investigate this, I have developed a novel adaptation of fluorescence recovery after photobleaching (FRAP), which, for the first time, allows us to assess incorporation of ECM protein during growth in live, intact *Drosophila* larvae. As such, recovery of fluorescently tagged proteins is a proxy for addition or relocation of ECM protein. We focus on Collagen IV (Viking), a conserved protein and major constituent of the basement membrane (BM). We have established a time

course for Vkg-GFP fluorescence recovery in both the heart and somatic body wall muscle at different developmental stages, under normal conditions and those in which *mmp2* is overexpressed. In wildtype, we report a strong phasic pattern of Vkg accumulation at second to third instar ecdysis (molting), potentially to support growth of the succeeding instar. Heart-specific overexpression of *mmp2* leads to significantly higher recovery of Vkg-GFP fluorescence, whereas overexpressing *timp,* the inhibitor of *mmp2,* in the heart leads to decreased Vkg recovery. This suggests that MMPs are positive regulators of Vkg/Col IV turnover in the ECM, which is in alignment with other recent studies (Davis et al., 2022; Töpfer et al., 2022). These findings have implications in not only cardiac conditions but also in other ECM-related disorders and diseases such as connective tissue disorders, muscular dystrophy, fibrosis, and cancer. Our observed changes in Vkg addition will be compared to changes in candidate gene expression. Supported by NSERC.

569T **Quantifying the relative contribution and furthering qualitative understanding of *ftz cis*-regulatory elements in *Drosophila melanogaster*** Matthew D Fischer, Patricia Graham, Leslie PickEntomology, University of Maryland, College Park

Embryonic development is coordinated by interactions within gene regulatory networks. This process is orchestrated at the level of transcription through the regulatory properties of enhancers, which direct spatiotemporal expression patterns when bound by specific *trans*-acting factors. Though enhancers can act upon promoters located at great distances irrespective of orientation, the contributions from these *cis*-regulatory elements (CREs) are limited by insulators and/or tethering elements that organize chromatin architecture. Much research has been conducted towards understanding the regulation of expression of the segmentation genes that pattern the basic body plan of the fruit fly, *Drosophila melanogaster*, during embryogenesis. The pair-rule genes (PRGs) of this pathway, such as *fushi tarazu* (*ftz*), are expressed in seven alternating stripes across the embryo. These PRGs are required for the development of body segments, and the mis-expression in a single transcriptional domain can result in the loss of a segment. I have investigated the *ftz* CREs to precisely determine their sufficiency to direct expression within *ftz* stripe domains and their necessity for doing so in the native context of the gene. To investigate the sufficiency, I generated 36 standardized reporter transgenes from 18 CREs, tested in both forward and reverse orientations. All CREs examined were inserted into the same XbaI site of the reporter plasmid, and the transgenes were inserted into the same genomic region. Through *in situ* hybridization experiments, I have determined that the qualitative patterns conferred by each of the CREs is orientation-dependent, and I have identified two putative insulators and/or tethering elements, proposed to explain this observation. To investigate their necessity, I targeted four genomic regulatory regions for precise deletion using the CRISPR/Cas9 system to generate seven deletion mutants. Though deletions were expected to cause lethality, most of the mutants are homozygous viable and fertile; only a mutant simultaneously removing two seven-stripe CREs was homozygous lethal. Quantitative gene expression analysis by fluorescent *in situ* hybridization chain reaction revealed that there is a critical threshold of *ftz* abundance required in each stripe for segmentation to proceed. From this work, I draw four conclusions: first, the *ftz* CREs are redundant and function together in a non-additive manner. Second, early stripe establishment is compensated by late stripe dynamics. Third, there is a critical threshold at which the analog information of gene expression converts to the digital information of segmental fate. Finally, while reporter transgenes can determine the sufficiency of a genomic region to direct specific spatiotemporal expression patterns, genomic edits in the context of a gene are required to determine their necessity for endogenous expression.

570T **The Role of Akirin/NuRD Interactions During Heart Development** Mia N Jones, Scott J NowakMolecular and Cellular Biology, Kennesaw State University

Congenital heart defects are the most prevalent birth defect in humans, with an occurrence as high as 1% of live births. Gene combinations and chromosomal changes are known to play a crucial role in the development of congenital heart defects, but the precise genetic and environmental factors involved in this process remain poorly understood. Cardiac malformations have been reproduced in many different experimental animal models by manipulating the expression of different genes functioning in the cardiac morphogenesis developmental pathways.The Nowak Lab has discovered several protein-encoding genes and novel regulatory proteins critical for embryonic heart development in *Drosophila melanogaster.* These proteins can help in identifying a potential link to congenital heart defects in humans.

During embryogenesis, the heart of *Drosophila melanogaster* develops into a two-chambered tube. The genes, gene products, and morphogenetic events directing this process remain consistent in both insects and humans. In *Drosophila*, the process of heart specification and formation is entirely controlled by a set of transcription machinery that works together with the transcription co-factor Akirin to mediate cardiac gene expression. By working together with the Nucleosome Remodeling and Deacetylase (NuRD) complex, Akirin is able is regulate gene expression. Embryos bearing

mutations in different NuRD subunits produce hearts that are severely misshapen, poorly patterned, and have reduced numbers of cardiomyoblasts in the finished organ.

The Nowak lab used live confocal-based imaging to assess whether mutant Akirin and NuRD complex subunit hearts, are capable of coordinated contraction and/or blood flow. We find that while mutant hearts do form, they bear several physiological and patterning defects. In addition to these genetic data, we have been further exploring the protein-protein interactions between the Akirin transcription cofactor and other subunits within the NuRD complex. The results from this investigation strongly suggest the presence of a protein-protein interaction between Akirin and NuRD complex deemed critical for heart patterning and development.

571T **Pointing us in the right direction: the *Drosophila melanogaster* wing as a model of planar cell polarity** Jessica Baker[1], Chase Holland[2], Natalya Tropea[2], Hanna Dery[21]Biology, Taylor University, [2]Taylor University

In developing tissues, planar cell polarity (PCP) organizes neighboring cells within an epithelial sheet. In vertebrates, disruption of PCP can lead to disorganization of auditory hair cells, fluid buildup in the brain due to disrupted cilia, and misoriented cell division leading to cysts in the kidney. In the Drosophila wing, PCP signaling directs the orientation and parallel alignment of actin-rich hairs extending from the apical surface of each wing cell.

Drosophila wing hairs are bundles of actin that protrude from an apical cytoskeleton 'pedestal' of microtubules and actin. This structure is important for proper wing hair localization, formation, and structural integrity. Disrupting the cytoskeleton early in hair development results in multiple or branched wing hairs and abnormal positioning of the hair initiation site. However, it remains less clear if regulation of the cytoskeleton is required for continued development of PCP after hair initiation. This continued development includes a septate junction dependent process that maintains parallel alignment of neighboring hairs.

Myosins are actin-binding motor proteins that regulate actin dynamics in the cytoskeleton. Drawing together a dozen undergraduate summer and semester projects, I will share our progress testing if Drosophila myosins are required for wing hair PCP after hair initiation. Reducing individual myosins using wing-specific Gal4s reveals that multiple myosins are required for parallel alignment of wing hairs. Further studies using time-course experiments and loss-of-function mutants suggest that myosins work with the septate junction protein Gliotactin to mediate parallel alignment PCP.

572T **Beyond wings: Roles for *apterous* gene in gut development, feeding initation and adult survival** Cindy Reinger[1], Laura Blackie[2], Pedro Gaspar[2], Michèle Sickmann[1], Dafni Hadjieconomou[2], Markus Affolter[1], Irene Miguel-Aliaga[2], Martin Müller[11]Cell Biology, Biozentrum, University of Basel, [2]Faculty of Medicine, Institute of Clinical Sciences, Imperial Collage London

Apterous (*ap*) encodes a transcription factor of the LIM homeodomain family and is best known for its important role in D/V patterning of imaginal discs. ap^{null} flies display the following phenotypes: no wings and halters and precocious adult death. The precocious death syndrome is characterized by adult death within 72 to 96 hrs after eclosion, and correlates with female sterility and abnormal adipose tissue. Using *Drosophila's* powerful toolbox of classical genetics and modern methods, we have studied *cis*-regulatory elements of *ap*. We were able to map and characterize an *ap* regulatory region, the *Life Span Enhancer (LSE)*, which is required and sufficient to rescue all of the phenotypes associated with the precocious death syndrome. However, a model for the comprehensive explanation of this syndrome and the connection between the individual disorders is still missing. We are currently investigating the genetic and cellular basis of the precocious adult death phenotype. ap^{null} flies (and flies lacking *LSE*) do not excrete their meconium and are not able to defecate. Furthermore, the mutant flies barely eat, implicating that feeding initiation is also disrupted. We found that during metamorphosis, both mutant fly lines have dramatic defects in mid- and hindgut remodelling processes resulting in a bloated and shortened adult midgut and lack of all rectal papillae in the hindgut ampulla. At the conference, we hope to present a model about how the various phenotypes of the syndrome are connected to each other.

573T **An EP mutant for *snuts* slows larval growth and development in high ambient temperatures** Kwon Yong-JinSchool of Life Science, Gwangju Institute of Science and Technology

Global warming has increased the surface temperature of the earth by an average of 0.87°C over the past 100 years, and it continues to do so in much higher rates. This rise in temperature has impacts on our ecosystem and its players, including insects. Insects are cold-blooded animals whose physiological activities are controlled by the ambient temperature. Therefore, using fruit flies, a molecularly amendable model insect, we intend to identify the

molecular mechanism that accelerates the development of insects in a high-temperature environment. The growth and development of fruit flies are affected by changes in ambient-temperature. The molecular mechanisms underlying the ambient-temperature dependent growth rate changes remain unclear in animals. In order to study the difference in development rate by temperature, screening was conducted through pupariation assay at 30°C using 691 Genexel EP insertion lines. We isolated several EP mutants, which shows much slower larval development at 30°C, compared to the controls. In a higher resolution analysis, using DIAMonDS (The Drosophila Individual Activity Monitoring and Detection System), we found that EP mutant for *snuts* (shrunken nuts) shows significantly slower growth rate at 30°C, whereas it shows significantly faster growth rate at 25°C. snuts has been reported to be important for testis development by influencing the testis stem cell niche in Drosophila, and is known to induce JNK pathway activity in the imaginal disc of fruit flies. To further investigate molecular mechanisms underlying the function of these genes, we currently performed bulk RNA seq on the organs that affect larval growth and development cultured at 25°C and 30°C. Our study will contribute to a better understanding of the molecular mechanisms by which global warming-induced elevated ambient temperature affects the growth and developmental rates of poikilothermic animals.

574T **Histamine immunoreactivity in secondary cells of the male accessory gland in *Drosophila melanogaster* appears during late pupal development** Jurrien Wilson[1], Martin Burg[21]Cell & Molecular Biology, Grand Valley State University, [2]Biomedical Sciences, Grand Valley State University

The male accessory gland of *D. melanogaster* produces numerous proteins and peptides that contribute to the seminal fluid [(1)]. While many components of seminal fluid are released by the accessory gland's 'main cells', some are generated by 'secondary cells', located at the distal tip of the accessory gland. We have found histamine immunoreactivity in the vacuolar-like compartments (VLCs) of secondary cells [(2)]. Mutations in the *Hdc* gene, which block histamine synthesis, have been shown to eliminate this immunoreactivity and alter the post-mating response of female courtship receptivity [(3)]. However, *Hdc* expression has not been detected in accessory glands of adult males, determined through analysis of *Hdc*-Gal4 expression lines [(4)], analysis of a gene fusion between the *Hdc* transcriptional promoter region and GFP, or analysis of currently available RNAseq data [(5)]. Thus, either *Hdc* is expressed very briefly during pupal development (or newly eclosed adults) or *Hdc* is expressed elsewhere and histamine is taken up by secondary cells. To determine when histamine immunoreactivity first appears in secondary cells, flies that express GFP in secondary cells were generated by using Gal4 lines that express in secondary cells [(6)]. Male pupae were selected at the white pre-pupa (WPP) stage and incubated at 25°C until the selected time of pupal development which lasted until eclosion at ~104 hours. Pupae between 65 hours to 102 hours as well as newly eclosed males were examined for the presence of histamine immunoreactivity using histamine antibody. Histamine immunoreactivity first appears in the lumen of the developing accessory gland at about 98 hours of pupation and in the secondary cells by 98-100 hours. Histamine immunofluorescence became more concentrated in the secondary cell VLCs during the last 4 hours of pupation up through the first day after eclosion. These results provide some indication of the time period in which pupal development should be examined for *Hdc* expression in secondary cells as well as in associated tissues of the male reproductive system. JW was supported by a GVSU OURS Kindschi fellowship.

Citations:

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575T **The PI 4-kinase Four wheel drive promotes cell surface expansion during apical constriction-mediated tissue folding** Wei Chen, Victoria Bergstein, Bing HeBiological Sciences, Dartmouth College

During tissue morphogenesis, cells often need to promptly adjust their surface area to accommodate dynamic cell shape change. Mechanisms that facilitate cell surface expansion during rapid cell shape changes while maintaining cell membrane integrity are not well understood. We found that during *Drosophila* ventral furrow formation, an epithelial folding process, apical constriction-mediated apical-basal stretching of the cell ("cell lengthening") results in a ~25% increase in cell surface area due to lateral expansion. Through a candidate screen, we identified the *Drosophila* PI-4

kinase IIIβ Four wheel drive (Fwd) as an important regulator for cell surface expansion during ventral furrow formation. Depletion of Fwd results in severe defects in apical constriction-induced lateral membrane expansion. Furthermore, the stretching/expansion of the apical domain of the cells adjacent to the constriction domain ("flanking cells") is also strongly impaired in *fwd* deficient embryos. Through computer modeling, we predicted that restricting lateral and apical expansion would result in non-overlapping but additive effects on tissue shape change during ventral furrow formation, including abnormal furrow morphology during apical constriction and reduced final invagination depth. The combined phenotype predicted by the model well recapitulated the ventral furrow defects observed in *fwd* deficient embryos. Finally, we show that Fwd functions redundantly with another PI 4-kinase, PI4KIIα, to promote cell surface expansion during *Drosophila* cleavage. Together, our findings uncovered Fwd as an important regulator for cell surface expansion across different morphogenetic processes and illustrated how cell surface area regulation may impact tissue-scale shape changes.

576T **Planar polarized localization of the atypical cadherin Fat is dynamic and regulated by Frizzled in the eye disc** Jiahui Liu[1], Madhav Mani[2], Richard Carthew[11]Department of Molecular Biosciences; NSF Simons Center for Quantitative Biology, Northwestern University, [2]Department of Engineering Sciences and Applied Mathematics; NSF Simons Center for Quantitative Biology, Northwestern University

Cells require positional information in the plane to generate polarized structures or to move in certain directions. This is called Planar Cell Polarity (PCP). In the Drosophila eye, patterning begins at the morphogenetic furrow (MF) with ommatidia formation. Ommatidia develop into two chiral forms with opposing rotation, creating a mirror symmetric boundary at the dorsoventral midline, called the equator. This PCP pattern is established by two pathways, one involves the atypical cadherin Fat (Ft) and the other involves the serpentine receptor protein Frizzled (Fz). Previous studies suggested that the pathways function independently in eye PCP. However more recently, other studies indicated that the Ft pathway acts upstream of the Fz pathway. To resolve this issue, we measured Ft intracellular localization in eye cells. Surprisingly, Ft localization is coordinately polarized along the plane of the tissue in a dynamic fashion. Anterior to the MF, cells are strongly polarized with Ft protein enriched at the cell interfaces facing anterior and polar. Cells in the MF strongly localize Ft at the cell interfaces facing the two poles and away from the equator. Ommatidial cells posterior to the MF localize Ft at the interfaces facing the posterior. This coordinate Ft polarization was disrupted in Fz mutant discs. Anterior to the MF, Ft polarization towards the anterior was still observed in Fz mutants but its preference for the poles no longer existed. Ft polarization was randomized in the MF of Fz mutants. Therefore, our results reveal evidence that Fz regulates the coordinate localization of Ft aligned to the dorsoventral axis. Another cadherin, Dachsous (Ds), has a graded expression in the MF, high at the poles and low at the equator. We found no significant difference in Ds expression between wildtype and Fz mutants, which suggests that Fz regulates Ft PCP by another mechanism rather than by acting on the Ds gradient.

577T **Regulation of localization and abundance of the Dachs-Approximated-Dlish complex by the protocadherins Fat and Dachsous** Hitoshi Matakatsu, Richard FehonMGCB, University of Chicago

A fundamental question in developmental biology is how size and cell number in organ is regulated during development. The Hippo signaling pathway regulates organ size by restricting activity of the transcriptional co-activator Yorkie, which promotes growth. It is known that several independent upstream inputs regulate Hippo signaling. Fat (Ft) and Dachsous (Ds), which act as receptor and ligand pair, together function to promote upstream Hippo pathway activity, thereby restricting growth. Loss of Ft leads to overgrowth and stabilization of the key effector Dachs, an unconventional myosin. Two additional regulators, a palmitoyltransferase Approximated (App) and SH3 domain containing protein Dachs Ligand with SH3s (Dlish) are required for Dachs stabilization and localization at the junctional cortex. Together these proteins form a 'core complex' that functions to promote growth. Current models for Ft-Ds signaling emphasize that Ft represses the core complex by preventing its cortical localization and promoting its degradation, while Ds promotes core complex activity by recruiting it to the junctional cortex. However, this model appears inconsistent with the observation that *ds ft* double mutants have a much stronger overgrowth phenotype than either single mutant alone, which suggests that Ft and Ds work synergistically to repress growth.

To understand how Ft-Ds signaling regulates the core complex, we have focused on the intracellular domains of Ft and Ds since these domains interact with the core complex *in vitro*. We used CRISPR-Cas9 to delete the entire ICD of Ft and Ds. To examine the localization and abundance of the core complex in vivo, we epitope tagged App with CRISPR-Cas9. We find that core complex proteins strongly co-localize in punctate structures at the junctional cortex. Deletion of the Ft ICD leads to overgrowth and accumulation of Dachs at the junctional cortex. In contrast, removing the Ds ICD leads

to reduction of core complex proteins and undergrowth of the wing. Pulse-chase experiments show that core complex proteins are stably maintained in junctional puncta, suggesting that these puncta are active signaling sites. These results are consistent with current models suggesting that Ds promotes core complex activity by recruitment to the cortex. However, we additionally find that in the absence of Ft removal of the Ds ICD has the opposite effect and instead causes even greater overgrowth than *ft* mutants alone. In addition, loss of Ds and Ft result in even greater accumulation of Dachs at the junctional cortex than seen in loss of Ft alone. Taken together, our results indicate that both Ft and Ds can function to either repress or promote growth in a context dependent fashion and that these activities are mediated by their intracellular domains.

578T **Role of Broad in regulation of proper border cell migration during *Drosophila* oogenesis** Daylon Douglas[1], Belen Ramos[1], Allison Jevitt[2], Dongyu Jia[11]Georgia Southern University, [2]Oklahoma Medical Research Foundation

Drosophila development is a highly regulated process by the precise timing and spatial cues of hormones and signaling pathways. The follicular epithelium of *Drosophila* egg chamber during oogenesis has been a well-studied model system to learn the morphological changes and cell migration. Broad (Br), a zinc finger transcription factor, is a well-known early response gene of the steroid hormone ecdysone which is essential for the initiation of metamorphosis. Our recent findings have shown that Br is a target of Notch signaling, and its downregulation is required for proper epithelial cell cuboidal-to-squamous transition during *Drosophila* oogenesis. Here, we report that proper border cell migration requires downregulation of Br during *Drosophila* oogenesis. Br expression can be induced by Notch signaling as early as stage 4. We found that Br expression decreases in border cells during the cell migration at stage 9. Further findings suggest JAK/STAT and ecdysone signaling are highly expressed during the onset of border cell migration, and their regulation leads to Br downregulation for proper border cell migration. In addition, we used single-cell RNA sequencing data to highlight the shift in gene expression which occurs as Br is suppressed and cells become mobile. Our gene ontology analysis of the gene sets discovered the enriched biological progresses, including cell migration, adherens junction organization and cell-cell signaling.

579T **Nutrient-driven dedifferentiation of enteroendocrine cells promotes adaptive intestinal growth** Hiroki Nagai[1], Luis Augusto Eijy Nagai[1], Sohei Tasaki[2], Ryuichiro Nakato[1], Masayuki Miura[1], Yu-ichiro Nakajima[11]The University of Tokyo, [2]Hokkaido University

Organ resizing improves the fitness of mature adult organisms that must survive in constantly changing nutrient environments. Although stem cell abundance is a fundamental determinant of adaptive resizing, our understanding of its underlying mechanisms remains largely limited to the regulation of stem cell division. Here we demonstrate that nutrient fluctuation induces dedifferentiation in the *Drosophila* adult midgut to drive adaptive intestinal growth. The early adult midguts rapidly grow in a feeding-dependent manner after eclosion. From in vivo lineage tracing and single-cell RNA-sequencing, we identify a subpopulation of enteroendocrine cells (EEs) that convert into functional intestinal stem cells (ISCs) in response to dietary nutrients. Genetic ablation of EE-derived ISCs severely impairs ISC expansion and midgut growth despite the retention of resident ISCs. Notably, starvation-refeeding cycle also induces the EE-to-ISC conversion in mature adults, indicating that EE dedifferentiation generally occurs in response to nutrient fluctuation. Our findings uncover a physiologically-induced dedifferentiation that ensures ISC expansion during adaptive organ growth in concert with nutrient conditions.

580T **Transcriptional co-repressor Atrophin regulates Hippo pathway target genes** Deimante Mikalauskaite[1], Cordelia Rauskolb[2], Tom Lehan[2], Srividya Venkatramanan[2], Mayank Chauhan[2], Derrick Michell[2], Kenneth Irvine[21]Waksman Institute, Rutgers University, [2]Department of Molecular Biology and Biochemistry, Waksman Institute, Rutgers University

The Hippo signaling pathway controls expression of growth-promoting target genes through its downstream effector, the transcriptional co-activator protein Yorkie (Yki). Hippo signaling removes Yki from the nucleus, under these conditions transcription of Hippo pathway target genes is repressed by the transcriptional co-repressor Tgi. Studies of Tgi suggest additional transcriptional repressors of Hippo pathway target genes exist. We have been investigating the transcriptional co-repressor Atrophin as a candidate co-repressor that contributes to Hippo signaling. Atrophin has multiple roles during Drosophila development. Using gene knock down and overexpression approaches, we have found that Atrophin regulates multiple Hippo pathway target genes in wing imaginal discs. Depletion of Atrophin results in elevated levels of Yki target genes in the middle of the wing pouch. Additionally, knockdown of Atrophin can partially suppress the reduction of target gene expression observed in Yki knockdown cells. In contrast, in the periphery of the wing pouch Atrophin knockdown decreases the expression of Yki target genes. Overexpression of Atrophin increases

expression of the same target genes in the proximal wing. Since Atrophin functions as a transcriptional co-repressor, these observations suggest that Atrophin could be directly repressing expression of Yki target genes in the distal wing, but indirectly activating them in the proximal wing. We are now identifying the mechanisms by which Atrophin exerts its effects on Hippo signaling and the regulation of Yki target genes.

581T **Macroglobulin complement-related protein is required late in Drosophila oogenesis to maintain egg elongation through cell shape changes and secretion of eggshell components** Lydia Bruno[1], Haifa Alhaydian[2], Robert Ward[11]Biology, Case Western Reserve University, [2]University of Kansas

Polarized epithelia engage in morphogenetic movements during development in all organisms. One such model system is the *Drosophila* ovary. The *Drosophila* ovary is composed of ovarioles possessing a string of progressively maturing egg chambers. Each egg chamber is defined by a monolayered somatic epithelium (the follicular epithelium), which encapsulate a 16-cell germline cyst. Several morphogenetic events occur in the follicular epithelium, including border cell migration, egg elongation, and morphogenesis of the dorsal appendages. A primary interest in our lab is studying the role of septate junction (SJ) proteins during morphogenesis. In *Drosophila* and other invertebrate organisms, the SJ is established and maintained by over 30 genes, and provides an occluding function to ectodermal epithelia, much like the tight junction in vertebrates. We have studied the role of SJ proteins during dorsal closure in embryogenesis, where we have shown a requirement for them in cell shape changes during the later stages of closure that is likely independent of their role in forming an occluding junction. We have previously demonstrated that SJ proteins are expressed in ovarian follicle cells. Using RNA interference, we have also shown that reducing SJ protein function results in round egg chambers, defects in border cell migration and aberrant dorsal appendages. Here, we demonstrate through use of Gal4 lines that are expressed ubiquitously in follicle cells beginning at different stages of oogenesis that the SJ protein Macroglobulin complement-related (Mcr) is required late in oogenesis for maintaining elongated egg chamber shapes. Consistent with these observations, early events in egg elongation including follicle cell rotation and polarized secretion of ECM components occur normally in *Mcr-RNAi* egg chambers. In contrast, late stage (st. 12) *Mcr-RNAi* egg chambers show defects in follicle cell shape, actin stress fiber organization, integrin expression, and eggshell integrity. Transmission electron microscopy reveals defects in the formation of the wax layer in *Mcr-RNAi* egg chambers. Traditional clonal analysis of loss of function *Mcr* alleles, and flip-out *Mcr-RNAi* clones are being used to address the cell autonomy of these mutant phenotypes.

582T **Transcriptome analysis reveals temporally regulated genetic networks during border cell collective migration** Emily Burghardt[1], Jessica Rakijas[1], Antariksh Tyagi[1], Pralay Majumder[2], Bradley J.S.C. Olson[3], Jocelyn A. McDonald[31]Kansas State University, [2]Presidency University, [3]Biology, Kansas State University

Collective cell migration underlies many essential organismal processes, including sculpting organs during development, wound healing in the adult, and cancer metastasis. *Drosophila* border cells, which undergo collective cell migration during normal development, are a genetically tractable model in which to study molecular drivers of collective cell migration. In the ovary, a group of 6-10 follicle cells are specified as border cells. At mid-oogenesis, border cells round up as a cluster, detach from the underlying epithelium and begin their migration. The cluster first extends directed actin-rich protrusions to move rapidly through the surrounding tissue. Later, as migration slows, the cluster rotates several times, then stops at the oocyte border. Successful border cell migration relies on cell signaling, cluster polarization, actin cytoskeleton remodeling, and maintenance of adhesion between border cells and with the nurse cell migratory substrate. Signals from ecdysone, JAK/STAT, EGFR/PVR, and other pathways initiate and direct border cell migration. Downstream targets of these signaling pathways, however, are poorly characterized. Nor is it known which genes, if any, are differentially expressed during distinct migration stages. To identify genes whose expression changes during border cell migration, we performed RNA-sequencing on slow border cells (*slbo*)-expressing border cells isolated at pre-, mid-, and late-migration stages. Transcriptome analyses of these cells identified 1,794 transcripts (1,394 unique genes) that were significantly differentially expressed during border cell migration. Downstream analyses, including gene clustering by expression patterns and testing for gene ontology enrichment, identified nine groups of genes with similar expression patterns. Many of these genes have known roles in cell junction assembly, epithelial differentiation, the actin cytoskeleton, and epithelial-to-mesenchymal transitions. Additional gene ontology and interaction analyses identified unexpected differential expression in genes that regulate ribosome biogenesis and immune signaling. We confirmed the expression and/or function of a subset of differentially expressed genes in border cells, further validating the RNA-sequencing approach. Thus, our transcriptome analysis revealed differentially expressed genes in migrating border cells and highlighted multiple genetic networks, as well as individual genes, that may function in border cell migration.

583T **Shining Light on Calcium-Mediated Morphogenesis: Forward Engineering Organ Development with Optogenetics and Piezo** Mayesha Sahir Mim, Jeremiah ZartmanChemical and Biomolecular Engineering, University of Notre Dame

Cells communicate to coordinate cellular processes across tissues, and calcium (Ca^{2+}) ions are second messengers facilitating such multiscale coordination. Ca^{2+}-selective cation channels mediate Ca^{2+} influx from the extracellular environment into the cell cytosol upon activation. However, current understanding of the biological mechanisms precludes direct control of Ca^{2+}-mediated processes and further drug screening to ameliorate diseases caused by Ca^{2+}-level anomalies. In this work, optogenetic and mechanosensitive channels, CsChrimson and Piezo, respectively, are utilized as tools along with drugs that affect their activity to investigate how Ca^{2+}-signaling dynamics impact epithelial growth and morphogenesis. Toward this end, we outline the phenotypic results of controlled stimulation of CsChrimson and dmPiezo. *Drosophila melanogaster* was used as an in vivo system, whose wing is acutely sensitive to changes in developmental pathways. We demonstrate that these channels regulate cytosolic Ca^{2+} dynamics, which can impact the activities of several downstream proteins and growth-related biological pathways. We show that these proteins, in turn, modulate cellular processes involved in morphogenesis, such as increased cell death in wing imaginal discs for optogenetic activation for longer periods (>6 hours) and for genetic overexpression of dmPiezo, and the morphology of the terminal organ reflected as severe phenotypes in adult wings. These activations also triggered compensatory proliferation by resulting in concurrently increased proliferation. For CsChrimson, such phenotypes proved to be directly correlated with the gradual increase in the intensity of light and consequently the concentration of cytosolic Ca^{2+}, both qualitatively and quantitatively. In pursuit of reverting these phenotypes, we are performing pharmacological reduction of the level of cytosolic Ca^{2+} by antagonizing other Ca^{2+}-pumping tools e.g. GPCR and IP3R, or by agonizing Ca^{2+}-removing channels e.g. Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA). We hypothesize that by controlling Ca^{2+}, the growth of cells and tissues can be directly controlled and the optimum level of cytosolic Ca^{2+} signaling to promote growth can be established. Such “Goldilocks zone” of Ca^{2+} level can be determined by systematically varying the width-half-max of optogenetic activation, e.g., by using a lower intensity of light or a faster optogenetic activation cycle. We used optogenetic and mechanosensitive channels and relevant drugs to forward engineer growth control in epithelia as a bottom-up approach toward tissue engineering. These findings regarding how Ca^{2+} signaling dynamics tune the robustness of organ growth and cellular processes provide scope for future applications related to phenotypic drug screening assays and innovation in treatments of human diseases.

584T **Analysis of pMad and Medea Expression in BMP Pathway in S2 Cells with Multiple Fluorescent Proteins** Hung-Yuan (Zeke) Chen, Gregory ReevesTexas A&M University

Morphogen gradients are important in early Drosophila embryo development. One such gradient, the BMP/Dpp gradient, regulates developmental processes such as patterning in the early embryo, which induces a series of protein interactions. On the dorsal side of the early embryo, the BMP morphogen Dpp activates Type I receptor Thickveins (Tkv), and Type II receptor Punt, which then phosphorylate Mothers against Dpp (Mad). Phosphorylated Mad (pMad) then binds to the cofactor Medea and the complex translocates to the nucleus to regulate BMP target genes. To investigate the dynamics of the BMP signaling pathway, we tag multiple fluorescent proteins on Mad and Medea to locate their positions during embryo development. Their expression depends on the morphogen concentration which is determined by the cell location. In this work, we tagged Mad and Medea with eGFP and mScarlet-I and transfected the construct into S2 cells. We used a confocal microscope to capture images of S2 cells before and after the addition of Dpp ligand. After adding Dpp into the system, we observed the migration of fluorescently-tagged Mad into the nuclei. Next, we plan to use optogenetic methods to deactivate the signaling pathway by fusing CRY2 sequence with Mad. We will inject those fluorescent constructs into flies and do image analysis of 14-cycle embryos to see the expression of these proteins and construct dynamic models based on the experimental data.

585T **Slowing Follicle Cell Migration Can Tune the Mechanical Properties of the Basement Membrane During Egg Chamber Elongation** Mitch Anderson[1], Sally Horne-Badovinac[1,21]Committee on Development, Regeneration, and Stem Cell Biology, University of Chicago, [2]Department of Molecular Genetics and Cell Biology, University of Chicago

Basement membranes (BMs) are specialized extracellular matrices found at the basal sides of epithelia. Dynamic changes to the architecture of the BM are critical for the morphogenesis of some tissues; however, the extent to which BM mechanics can be tuned to produce different morphogenetic outcomes is unknown. The Drosophila egg chamber is a tractable system to study how tissues change their BM architecture and how BM architectural dynamics influence tissue shape. The egg chamber is comprised of an inner germ cell cyst, surrounded by a monolayer of epithelial cells called

follicle cells, and an overlying BM. As the egg chamber develops, it transitions from a spherical to an elongated shape. The BM architecture changes as the egg chamber begins to elongate; prior to elongation, the BM is largely uniform, and later, polarized BM 'fibrils' are added to the matrix via the collective migration of the follicle cells. The polarized BM is thought to act as molecular corset to mechanically channel egg chamber growth along one axis and thereby lengthen the tissue. If the follicle cells do not migrate, BM fibril formation fails, and egg chamber elongation is perturbed during early stages. However, by studying a mutant condition in which follicle cell migration is both delayed and slowed, we have found that the egg chambers instead have an elongation defect that is both milder and that manifests at a later stage - when the nurse cells dump their contents into the oocyte. We are now exploring how these migratory changes affect the mechanical properties of the BM and the idea that nurse cell dumping presents a particularly strong challenge to the ability of the BM corset to control tissue shape.

586T **Characterization of *kayak* (*kay*) mutant phenotypes in *Drosophila melanogaster* eye development** Manuel A Zuniga-Garcia[1], Juan R Riesgo-Escovar[21]Developmental Neurobiology, Instituto de Neurobologia, UNAM, [2]Instituto Neurobiologia, UNAM

During development, *kayak (kay)* is a pleiotropic gene transcribed in many places and stages. *kay* participates in several different signaling pathways. We characterize the eye phenotypes of four *kay* alleles. To do so, we generated mutant clones for *kay[1], kay[2], kay[4],* and *kay[5]* in the eye as these alleles are all embryonic lethal. We analyzed them by optical, confocal, and scanning electron microscopy (SEM). We describe adult mutant phenotypes. Mutant *kay[1], kay[2],* and *kay[5]* clones exhibit ommatidium and bristle defects. Moreover, mutant *kay[5]* shows big cuticule indentations and loss of ommatidial structure in the medial-anterior eye region. Semithin optical sections analysis revealed ommatidium polarity defects and ommatidia with fewer photoreceptors. Finally, we found that the rate of mutant versus wildtype photoreceptors decrease in *kay* clones. Thus, Kay is necessary for cell fate / photoreceptor survival. Taken together, these results indicate that *kay* plays several key roles in general eye development.

587T **Identification of CryAB as a NUAK kinase substrate that influences protein aggregation in muscle tissue** Ziwei Zhao[1,21]Biochemistry & Molecular Biophysics, Kansas State University, [2]Kansas State University

NUAK belongs to the AMP-activated protein kinase (AMPK) family, which is comprised of conserved serine/threonine protein kinases known to regulate a wide variety of biological processes, including glucose transport, lipid metabolism, and tumor suppression and promotion. Previous results in our lab have shown that a mutation in *Drosophila* NUAK results in the degeneration of larval body wall muscles. Prominent features of these muscles include the abnormal accumulation of select proteins, such as Filamin and CryAB, and an increase in the autophagic markers p62 and Atg8a, suggesting that autophagy is compromised. To understand how kinase activity contributes to NUAK function, we mutated a key residue important for catalytic activity using CRISPR/Cas9. This kinase domain mutation resulted in thinner muscles and lethality before the end of the first instar larval stage. To circumvent this early lethality and assess how the loss of NUAK kinase activity affects contractile muscles later in larval development, we turned to transgenic mutagenesis approaches. Dominant expression of three independent mutations proposed to abolish enzymatic function all resulted in abnormal protein accumulation and defective sarcomere patterning, thus establishing a role for NUAK kinase activity in contractile muscles. To uncover substrate proteins that may be phosphorylated by NUAK, we performed a yeast two-hybrid screen. *l(2)elf*, which encodes for CryAB, emerged as a top candidate since mutations in human CryAB/α-crystallin B cause a type of protein aggregate disease called Myofibrillar Myopathy. Our results thus far show that overexpression of NUAK increases CryAB phosphorylation and this post-translational modification can be reversed with phosphatase treatment. To identify which residue(s) of CryAB are phosphorylated by NUAK, we performed mass spectrometry experiments and observed increased phosphorylation on two serine residues in CryAB (S68 and S70). Mutation of either serine to alanine abolished phosphorylation, thus confirming the locations of these post-translational modifications. Experiments are underway to test the functional importance of these newly identified phosphosites and their relevance to NUAK-mediated protein accumulation.

588F **Fat expression dynamics and growth control in the developing *Drosophila* wing pouch** Andrew E Liu, Richard CarthewDepartment of Molecular Biosciences, Northwestern University

One fundamental aspect of developmental biology is growth control – how cells learn to stop proliferation when the organism or an organ reaches its optimal size. Loss of growth control is found in diseases such as cancer. The Drosophila wing is a model system for growth control, where growth is spatially homogenous and exponential during larval stages, and growth ceases at the larval-pupal transition. Previous research found that two proto-cadherins, called Fat and

Dachsous, are expressed in all wing cells where they contribute to growth control. Loss of either Fat or Dachsous results in overgrowth of the wing. The cadherin proteins are localized to the cell membranes where they bind to one another and regulate the Hippo pathway. But the molecular mechanism by which they control growth is poorly understood. To understand the nature of how they regulate growth, we measured endogenous Dachsous and Fat protein levels across the growing wing. The proteins are expressed in global gradients that oppose one another; Dachsous expression is highest at the wing margin and lowest at the wing center, while Fat is highest at the center and lowest at the margin. The Dachsous gradient is under transcriptional control, whereas the Fat gradient is formed by an RNA-independent mechanism. The Dachsous gradient scales with wing size as it grows over time, while the Fat gradient shallows as the wing approaches its final size. When Dachsous is absent, shallowing of the Fat gradient is temporally delayed, and the final wing size is abnormally large. Our model is that Dachsous-Fat protein interactions shape the global gradient of Fat, which then promotes wing cell proliferation. When the gradient flattens, then cells no longer receive a proliferation signal and they stop growing. We are currently working on building genetic variants that test this model.

589F ***Drosophila eEF1a2* has a cell autonomous role in the actin-fiber maintenance of adult muscle** Hidetaka Katow, Hyung Don RyooCell Biology, New York University

eEF1a1 and eEF1a2 are translation elongation factors that deliver amino acyl-tRNAs to ribosome for mRNA translation. In addition, these proteins are thought to have non-canonical functions unrelated to mRNA translation. While eEF1a1 is expressed ubiquitously, eEF1a2 expression is limited to specific cell types including muscles and neurons. The precise in vivo roles of these proteins remain unclear. To better understand *eEF1a2* function in *Drosophila*, we generated loss of function *eEF1a2* mutants using CRISPR/Cas9 gene editing. The mutants were homozygous viable. We confirmed through sequencing and qPCR that these flies are true loss of function mutants.

The newly eclosed *eEF1a2* mutants had no visible phenotypes, but by 10 days after eclosion, the mutants held their wings in abnormal angles. This prompted us to examine the indirect flight muscles (IFM), which regulate wing movements. Immunohistochemical analysis of IFM revealed that mutant muscles had myofibrils that were approximately half as thick compared with control flies.

To assess if the wing phenotypes became worse with aging like some muscle diseases, we dissected aged flies (>30 days after eclosion). The results showed that actin was distributed unevenly in the aged IFM at this stage. Additionally, these flies generated trachea-ensheathing actin rich structures (Tears) in peripheral trachea.

These wing phenotypes were fully rescued by genomic duplications. Consistently, the IFM-specific knockdown of *eEF1a2* also caused an uneven actin distribution and mild Tears. These results indicate that *eEF1a2* has a cell autonomous role in muscle actin-fiber maintenance. Of note, *eEF1a2* loss did not affect muscles in the larva body wall, indicative of *eEF1a2's* role in specific cell types at specific stages of the life cycle.

590F **Integration of the *Sex combs reduced* Hox gene into the Drosophila leg gene regulatory network** Xinyuan Liu, Teresa OrenicBiological 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 also a TF, which is required for upregulated expression of both *Scr* and *ScrE-GFP.* Even though *bab* locus contains 2 paralogous gene, *bab1* and *bab2*, Bab2 plays a predominant role in distal repression of *Scr*. 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.

591F **Morphogenetic forces regulate Hippo signalling in epithelial tissues via basolateral spot junctions** Ben Kroeger[1], Sam Manning[1], Yoshana Fonseka[1], Kieran Harvey[1,21]Monash University, [2]Peter MacCallum Cancer Centre

Organ size is controlled by numerous factors including mechanical forces, which are mediated in part by the Hippo signalling pathway. In growing *Drosophila melanogaster* epithelial tissues, cytoskeletal tension influences Hippo signalling by modulating the subcellular localisation of key pathway proteins in different apical domains, namely adherens junctions, the sub-apical region and the medial apical cortex. Using a combination of molecular genetic approaches and high-resolution imaging, we have discovered the existence of basolateral spot junctions in *D. melanogaster* epithelial cells, and a key role for them in coupling morphogenetic forces and Hippo signalling. Like at adherens junctions, the kinase Warts is recruited to basal spot junctions via Ajuba and E-cadherin, which prevent Warts activation by segregating it from upstream Hippo pathway proteins. Modulation of cytoskeletal tension more profoundly impacts basal Warts than adherens junction-associated Warts, underscoring the importance of basal spot junctions for mediating the influence of mechanical forces on Hippo pathway activity. Basal spot junctions are distinct from focal adhesions but the latter profoundly influences the abundance of spot junctions by modulating the basal-medial actomyosin network and tension experienced by spot junctions. Thus, in growing epithelial tissues, E-cadherin-mediated spot junctions at the basal-most region of the lateral cell membranes are important for coupling morphogenetic forces to Hippo pathway activity and organ growth.

592F **The DUB complex increases Wingless/Wnt signaling strength by stabilizing Arrow/LRP6** Ghalia Saad Siddiqui[1], Zachary T. Spencer[2], Victoria H. Ng[3], Hassina Benchabane[2], Deepesh Duwadi[2], Ben Maines[2], Kai Yuan[2], Sara N. Kassel[4], Anant Mishra[3], Ashley Pimentel[3], Andres M. Lebensohn[5], Rajat Rohatgi[6], David J. Robbins[7], Ethan Lee[8], Yashi Ahmed[91]Department of Molecular and Systems Biology and the Dartmouth Cancer Center, Dartmouth, [2]Dartmouth, [3]Cell and Developmental Biology, Vanderbilt University, [4]Vanderbilt University, [5]3Laboratory of Cellular and Molecular Biology, National Institutes of Health, [6]Stanford University School of Medicine, [7]Department of Oncology, Georgetown University, [8]Department of Cell and Developmental Biology, Vanderbilt University, [9]Molecular and Systems Biology, Dartmouth

Colorectal cancer (CRC) is the second leading cause of cancer-related death worldwide. Nearly 100% of CRC cases are caused by aberrant activation of the evolutionary conserved Wnt/Wingless (Wg) signaling pathway, a pathway that is essential for animal development and tissue homeostasis. The Wnt ligand engages its receptors to trigger pathway activation. In normal cells, regulation of the Wnt receptors determines the level of signaling, whereas receptor overactivation drives several types of cancers. Therefore, control of Wnt receptor activity is critical for proper signaling levels, but the mechanisms mediating receptor regulation are incompletely understood. We recently identified a deubiquitinase complex (DUB) comprised of three proteins, USP and two WD40 repeat-containing proteins, that increase levels of the Wnt receptor Arrow/LRP6 and promote Wnt signaling *in vivo*. By reducing Arrow/LRP6 turnover, the DUB complex enhances the sensitivity of target cells to Wingless stimulation, thereby increasing the amplitude and spatial range of their signaling responses. Inactivation of the DUB complex results in a cascade of causal signaling defects in Wingless-responding cells in the adult intestine: Arrow/LRP6 levels are decreased, Armadillo/β-catenin stabilization is diminished, Wingless target gene activation is attenuated or abolished, and the concentration-dependent regulation of Wingless responses is lost. Consequently, Wingless-directed patterning and tissue homeostasis are disrupted. These results, identify an evolutionarily conserved LRP6/Arrow stabilization mechanism and reveal its crucial role in the concentration-dependent activation of signaling throughout the spatial range of the Wingless morphogen gradient.

593F **The different facets and functions of Wingless (Wg) signalling in *Drosophila* ovarian follicle cell migration** Poulami ChatterjeeLife Sciences, Presidency University, Presidency University

From gastrulation to organogenesis, as well as being linked to morphogenesis and wound healing, collective cell migration (CCM) is essential for several developmental processes. As opposed to that, erroneous cell motility might accelerate the spread of metastatic cancer and inflammatory illnesses. The *Drosophila* egg chamber (EC) starts as a spherical tissue during early ovary development which with maturation elongates to create an elliptical egg shape. A sheet-like, direction-independent, specialised migration of the outer follicular epithelial cells creates the molecular corset that gives a mature egg chamber its elliptical form. Follicle cells migrate by forming protrusions, much like any other migrating cell but unlike other CCM, neither a leader cell nor a known source of cues for guidance are shown to exist. The primary focus of our research is to unveil the function of canonical wingless (mammalian Wnt) signalling in

this atypical follicle cell migration. It all started with performing the genetic screening of the canonical wingless pathway genes. We targeted every corner of the pathway i.e., the ligand, the receptor, the destruction complex, the effector genes and even the inhibitor gene that represses the transcription of wingless targeted genes. Our genetic modification, live imaging data revealed potential canonical wingless signalling pathway candidates that change the aspect ratio and hence the shape of the mature egg by altering the stochasticity of FC migration. We observed a distinct egg phenotype by knocking down the transcriptional effector molecule of wingless pathway i.e., *armadillo*. Additionally, we have noticed morphological changes in the follicular cells niche within the topologically closed epithelium. At low expression level, various participants of this canonical wingless pathway showed both rounder as well as protracted egg phenotype. However, a unique egg morphology was revealed when the nuclear localised transcription complex molecule *groucho*, which has a repressive function, was knocked down. Inferring from all of our experimental findings we can conclude that wingless signalling might have a substantial role in follicular cell migration and, consequently, egg chamber rotation.

594F **Transient epithelial folding and out-of-plane division are genetically patterned mechanical sinks that release compressive stress resulting from tissue tectonic collision** Bipasha Dey[1], Verena Kaul[2], Girish Kale[2], Michiko Takeda[1], Yu-Chiun Wang[1], Steffen Lemke[21]RIKEN BDR, [2]Centre for Organismal Studies Heidelberg, University of Heidelberg

The cephalic furrow (CF) is an epithelial fold formed between the head and trunk ectoderm in the gastrulating *Drosophila* embryo. Active cell deformation that initiates the CF is precisely regulated by genetic patterning and mechanical self-organization. The precision and linearity with which the CF is formed suggests that it is under evolutionary constraint. However, unlike most other epithelial folds, the CF ultimately unfolds without generating a curved or internal tissue structure, and hence its function and evolution remain a mystery. We investigate tissue-scale morphodynamics and find that genetic or optogenetic ablation of the CF causes tissue buckling. Buckling occurs at approximately the same head-trunk boundary where the CF normally forms, albeit with a delayed timing. Abrogation of mitosis in the head or germband extension (GBE) in the trunk greatly reduces the persistence and depth of buckling. Head mitosis and GBE each generate a distinct, yet locally coherent tissue flow pattern that interfaces at the CF or the buckle, suggesting that collision of two mechanically distinct territories in the absence of CF leads to buckling, akin to tectonic collision. Buckling due to loss of CF increases the disorderness and reduces the linearity of tissue boundary, suggesting that the CF preempts buckling to prevent disruption of spatial patterning. Phylogenetic analysis reveals that the CF is an evolutionary novelty originated in the stem group of Cyclorrhaphan flies as a result of a shift in the anterior-posterior patterning. Representative non-Cyclorrhaphan species *Chironomus riparius* lacks the CF and employs an alternative strategy to prevent tissue buckling. Contrasting with *Drosophila* where the head mitosis is predominantly in-plane, widespread expression of the spindle-cortex adaptor protein Inscuteable (Insc) enables head mitosis to be mostly out-of-plane in *Chironomus*. Loss of Insc increases in-plane division, leading to buckling of the dorsal trunk tissue, suggesting that out-of-plane division can release compressive stress in *Chironomus*. Our results suggest that early fly embryos have evolved at least two mechanisms to release compressive stress that arises during gastrulation in the ectoderm. We propose that the CF was either evolved or co-opted as a genetically-patterned mechanical sink to prevent buckling in place of out-of-plane division.

595F **Twin roles of the zinc-finger transcription factor Castor: specification of cardiac cell subtypes and regulation of cardiac progenitor cell division** Abbigayle J. Gamble[1,2], Rajnandani Katariya[1,2,3], M. Rezaul Hasan[1,2,3], Melisa Spognardi[2,4], Kuncha Shashidhar[1,2], Mofazzal K. Sabbir[1,2], Alishba Rizwan[2,5], Manoj Panta[1,2], Andrew J. Kump[1,2,3], Shaad M. Ahmad[1,2,31]Department of Biology, Indiana State University, Terre Haute, IN, [2]The Center for Genomic Advocacy, Indiana State University, Terre Haute, IN, [3]Rich and Robin Porter Cancer Research Center, Terre Haute, IN, [4]Saint Mary-of-the-Woods College, Saint Mary-of-the-Woods, IN, [5]Department of Psychology, Indiana State University, Terre Haute, IN

Mutations in the zinc-finger transcription factor-encoding gene *CASZ1* lead to aberrant heart development in humans, Xenopus, and mice, indicating its conserved role in cardiogenesis. Our phenotypic analysis of a null mutation of *castor* (*cas*), the *Drosophila* ortholog of *CASZ1*, shows that *cas* has two distinct roles in heart development. First, *cas* is required for mediating all three categories of cardiac progenitor cell division: asymmetric, symmetric, and cell divisions at an earlier developmental stage. Second, *cas* prevents subsets of cells in the most anterior region of the heart, the anterior aorta, from becoming specified as *seven up*-expressing cardial cells (Svp-CCs). Svp-CCs are present in the posterior aorta and the even more posterior heart proper, regions of the heart determined by the expression of the Hox genes *Ultrabithorax* (*Ubx*) and *abdominal A* (*abd-A*). Intriguingly, both *Ubx* and *abd-A* repress *cas*, and ectopic expression of either of these two Hox genes in the anterior aorta leads to the ectopic specification of Svp-CCs there—a result which phenocopies *cas* loss-of-function mutants. Collectively, these data raise the possibility that that *Ubx* and *abd-A* specify Svp-CCs in the posterior aorta and the heart proper by repressing *cas* in those regions. In contrast, in the anterior aorta,

in the absence of both *Ubx* and *abd-A*, *cas* levels may be sufficiently high to repress the Svp-CC fate. We are presently testing this hypothesis for *cas*-mediated Svp-CC specification and attempting to elucidate the pathways through which *cas* regulates cardiac progenitor cell division.

596F **The counter-intuitive role of junctional tension during morphogenesis in the drosophila embryo** Thom de HoogUniversity of Zürich

During embryogenesis, cells collectively change their shape to facilitate the form and function of emerging tissues. This happens in a highly robust manner, suggesting the existence of mechanisms that assure appropriate tissue shape and integrity during morphogenesis. How emerging forces, that result from subcellular contractile networks, are integrated in space and time to coordinate organ formation, is largely unknown.

To unravel such mechanisms, we chose to study apical constriction in the amnioserosa during dorsal closure in Drosophila melanogaster. Amnioserosa is a tissue consisting of a single layer of approximately 200 cells that, by contraction, drives the convergence of two lateral epithelial sheets. During this process, two distinct force-generating actomyosin networks act concurrently: one at adherens junctions and another at the medio-apical surface. The current consensus is that the medio-apical network provides the main driving force for tissue contraction. The role of force provided by the junctional actomyosin network in this process remains unclear.

We overexpressed a selective inhibitor of junctional actomyosin that resulted in lower tension at the adherens junctions. As expected, junctional relaxation resulted in disordered cell shapes at the apical surface. However, we also observed an accelerated convergence of the lateral epithelial sheets. This unexpected result suggests that junctional tension interacts antagonistically with the force generated by the medio-apical actomyosin network.

These results point to the intriguing possibility that junctional tension plays a central role in regulating the shape and timing of apical constriction by enforcing regularity in cell shape. Antagonistically interacting forces may serve as a general mechanism during morphogenesis to robustly integrate emerging shapes, from cells to tissues over time.

597F **Optogenetic perturbation of a pre-gastrulation niche to modulate neural lineage fates** Kelli D Fenelon, Priyanshi Borad, Theodora KoromilaBiology, University of Texas Arlington

Cell differentiation, which drives embryonic development, is initiated by Pioneer Factors (PFs) that prime cells to shift their external roles by transforming their transcriptional landscapes. Pre-gastrulation, PFs 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 transcription factors involved in *Drosophila* embryonic brain development, such as Odd-paired (Opa)/ Zinc finger in the cerebellum (ZIC3) and Ocelliless (Oc)/OTX2, are largely conserved across the animal kingdom. Despite ongoing efforts, the synergistic functionality of transcription factor spatiotemporal dynamics and their rippling long-term effects are poorly characterized. Using a combination of optogenetic and RNA live imaging tools, we are able to directly manipulate PF protein levels within individual cells *in vivo* and visualize immediate, as well as delayed temporal effects in real time with 4D, super resolution specificity during development. We develop *in vivo* tools for optogenetic control of transcription factor function through use of iLEXY- and LINX-PF endogenous transgenic flies, which facilitate photo-inducible nuclear export, providing acute temporal control through rapid translocation and recovery. In addition, using super resolution microscopy we, visualize simultaneous active transcription of multiple downstream genes through the use of MS2 and PP7 stem loop:coat protein that allows us to observe RNA transcripts in real time with single-cell specificity. Here we demonstrate novel combinatorial, pioneering functions of Opa and Oc in the establishment of the neural cell lineage in *Drosophila* embryo. This work is vital to understanding the cell fate determinations which beget the coordinated cellular diversity of mature animalia.

598F **Discovery of novel short linear motifs in insect segmentation proteins** Minh Lê, Leslie PickEntomology, University of Maryland

Many examples in recent years have highlighted the importance of cis-regulatory changes in regulatory genes as drivers of morphological evolution. Changes in protein function may be less common because of pleiotropic effects of these changes, or they may be equally common in nature but less well-studied because they are more difficult to identify. Previous work from our lab showed that changes in short protein interaction motifs in the Hox protein FUSHI TARAZU (FTZ) switched its function during insect evolution from an ancestral homeotic role to pair-rule segmentation in lineages leading to *Drosophila* (Lohr et al., 2001; Lohr & Pick, 2005; Heffer et al., 2010). Here, we used MEME, a sequence

alignment-based motif discovery tool, to probe whether similar evolutionary variation in short linear motifs in other pair-rule proteins could be identified computationally. One of the changes we identified is the presence of an LXXML motif in the *Drosophila* pair-rule protein RUNT. This motif was not found in RUNT homologs from other insects such as *Bombyx mori*, *Tribolium castaneum*, *Oncopeltus fasciatus*, or in vertebrate RUNX homologs. This motif is highly conserved across different *Drosophila* species, as well as other non-drosophillid dipterans, including mosquitos. A predicted RUNT structure using Alphafold suggests that this motif is nested within the N-terminal alpha helix region of the Runt Domain. To test whether this motif is necessary for RUNT function, the UAS-GAL4 system will be used to ectopically express wild type or mutant RUNT protein lacking the LXXML motif (RUNT[ΔLXXML]) in *D. melanogaster*. Based on well-established assays for RUNT function (Aronson et al., 1997; Wheeler et al., 2002; Walrad et al., 2010), we will assess the role of this motif in segmentation. Additional mutagenesis will target individual amino acids within the LXXML motif, if it appears to be necessary for function. More broadly, progress in our understanding of protein structure-function relationships has improved our ability to predict protein activities from sequence information. This, coupled with the ability to carry out functional tests in *Drosophila* and an increasing number of new insect model systems, will allow us to objectively assess the importance of changes in protein function during insect evolution.

599F **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[1,2,3], Neal Jeffries[4], Shaad M. Ahmad[1,2,31]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]National Heart, Lung and Blood Institute, National Institutes of Health

The development of a complex organ requires the specification of appropriate numbers of its constituent cell types as well as their correct positioning within the organ. We previously showed that 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. Statistical analysis demonstrated that these positioning defects cannot be completely explained by steric constraints caused by differing number of cardial cells in contralateral hemisgments due to cell division defects. 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 Fox mutants 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: 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.

600F **Investigating a role for septate junction proteins in cell flattening during dorsal closure** Robert Ward, Oindrila De, Jae Ho LeeBiology, Case Western Reserve University

Septate junctions (SJs) form an occluding barrier in invertebrates. SJ genes are well known for their canonical barrier function, but some studies have highlighted a non-occluding role for SJ proteins in *Drosophila* morphogenesis. In our recent studies, we demonstrated a non-barrier requirement for core SJ proteins in dorsal closure (DC), which occurs during mid-embryogenesis to seal an epidermal gap resulting from germ band retraction. DC is driven by actomyosin-based contraction of the extraembryonic amnioserosa cells, tension at the leading-edge, and zippering at the canthi mediated by filopodial dynamics and adhesion of the leading-edge cells. The rate of closure is bimodal with an initial slow phase and a later fast phase. During closure, cells of the lateral epidermis elongate dorsoventrally to cover the gap that represents approximately 40% of the surface area of the embryo, but this also raises an interesting and unexplored aspect of DC. In order for the lateral epidermal cells to cover this expansive gap, these cells must substantially increase their apical surface area. Additionally, if the volume of the cells remains constant, increases in apical surface area must be accompanied by reductions in cellular height along the apical-basal axis. To test this hypothesis, we employed morphometric analysis and found that wild type cells significantly reduced their apical-basal height between the slow and fast phases of DC. We are currently investigating the correlation between apical surface area and apical-basal height during different stages of DC in wild type embryos. Interestingly, loss-of-function mutations in core SJ genes results in a failure to complete DC and is associated with inability of mutants to enter the fast phase of DC. Through live and fixed tissue analysis, we determined that SJ mutant embryos have significant defects in cell shape, including reduced area, a lower aspect ratio, and increased circularity of their apical surfaces in comparison to wild type embryos during later

stages of DC. SJ mutant cells are also significantly taller as compared to wild type cells at this stage, suggesting a defect in cell flattening.

601F **Investigating the role of ecdysone signaling in dorsal closure using Halloween genes.** Jae Ho Lee[1], Riti Mital[2], Amenda Li[2], Robert Ward[21]Case Western Reserve University, [2]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. There is also a less well-characterized pulse of 20E during mid-embryogenesis. 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. 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. *dib* and *sro* mutant 22-hr old embryos show nearly penetrant defects in dorsal closure characterized by uniformly large dorsal holes. The severity and penetrance of these defects suggested an early defect in dorsal closure. Surprisingly, we found that JNK and Dpp signaling appear normal in *dib* and *sro* mutant embryos and that the leading edge produces an actomyosin cable. We are using live imaging and immunostaining of fixed embryos to examine actomyosin dynamics and cell shape changes in *sro* mutant embryos. To get a better understanding of 20E signaling during mid-embryogenesis, we conducted RNA sequencing of wild-type and mutant 9- and 13-hour embryos and found approximately 2000 genes are differentially expressed between wild-type and Halloween mutant embryos. Sorting these genes by fold change, threshold levels, likely expression in the epidermis and existence of RNAi lines (that are not already shown not to have a phenotype when expressed in the dorsal epidermis) resulted in ~70 candidate genes that we are testing using RNA interference. In addition, among the top 50 20E-induced genes, 11 encode long non-coding (lnc) or antisense RNAs. Our initial approaches to addressing these genes include examining deficiencies that lack lncRNAs and excising local P-elements to generate specific deficiencies of them. We will characterize phenotypes in anticipation of using CRISPR to induce more precise mutants of candidate lncRNA genes for further investigation of their role(s) in dorsal closure.

602F **Malvolio, the *Drosophila* ortholog of human NRAMP2 metal ion transporter, is required for salivary gland morphogenesis** Rajprasad Loganathan[1], Srihitha Akula[2], Tony Zhu[2], Aditi Kulkarni[3], Rika Maruyama[3], Deborah J Andrew[21]Biological Sciences, Wichita State University, [2]Cell Biology, Johns Hopkins University, [3]Johns Hopkins University

Malvolio (Mvl) is a member of the SLC11 family of metal ion transporters, and is the Drosophila ortholog of the mammalian natural resistance-associated macrophage proteins (NRAMPs). Mvl shows Fkh-dependent expression during embryonic salivary gland (SG) morphogenesis. To study the role of *Mvl* in the SG, we generated a null allele of *Mvl* (*Mvl^{exc1}*), transgenic fly lines for expressing both GFP-tagged and untagged Mvl (UAS-Mvl, UAS-Mvl-GFP), as well as Mvl-specific polyclonal antisera. Our initial analysis showed no effect on viability in *Mvl* zygotic loss although developmental delay was observed in *Mvl^{exc1}/Df(Mvl)* with adults eclosing 48 – 72 hours later than their balancer-containing siblings. Zygotic loss of *Mvl* resulted in mild effects on SG morphology and small gaps in the denticles. Combined maternal and zygotic loss resulted in pronounced defects in SG morphology and increases in the frequency of the denticle gaps. We also observed a loose assemblage of CrebA+ embryonic cells in the anterior region of the *Mvl* homozygotes, which we have not identified. Collectively, these results demonstrate varying degrees of cell morphogenetic defects with the loss of *Mvl* during embryogenesis. Imaging of junctional proteins to investigate cell morphogenetic changes in *Mvl* null embryos revealed that the levels and localization of the adherens junction protein E-cadherin and polarity marker Bazooka were comparable to wild-type SGs, whereas 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 co-stained embryos with Mvl and several organelle-specific markers. These experiments revealed Mvl localization to Golgi, early and late endosomes. We hypothesize that the cell morphogenetic defects in *Mvl* loss of function is the likely result of 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.

603F **Shaping 3D geometry during tubulogenesis: the PDZ domain-containing protein Arc regulates Crumbs and myosin II during embryonic salivary gland morphogenesis** Ji Hoon Kim, Kwon Kim, Devin Vertrees, Rika Maruyama, Deborah AndrewCell Biology, Johns Hopkins University School of Medicine

The proper architecture of an organ is inseparable from its optimal functionality and physiology. During development, three dimensional tubular organs arise from two dimensional primordia through dynamic changes in cell shape and arrangement. By using embryonic salivary gland (SG) development as a model system to study tubulogenesis, we have discovered that the FoxA transcription factor Fork head (Fkh) is essential for tube formation and internalization. Thus, a subset of Fkh target genes should have roles in SG morphogenesis. *arc*, an early-expressed SG gene whose expression in the SG and other tubular epithelia requires Fkh, encodes a large cytoplasmic protein containing two PDZ domains. We have discovered that Arc contributes to overall SG dimensions; loss of *arc* results in shorter, stubbier SG tubes with more cells in circumference and Arc overexpression results in highly elongated SG tubes with fewer cells in circumference. Similar SG phenotypes were induced by perturbing function of non-muscle myosin II (MyoII) and its antagonistic transmembrane protein Crumbs (Crb) in SG. Both the hyperactivation of MyoII and the suppression of Crb activity resulted in shorter SGs with more cells in circumference, as seen in *arc* loss-of-function mutants. Moreover, Crb levels were significantly reduced in *arc* null mutant SGs and Arc overexpression resulted in the mis-localization of Crb protein. Accordingly, MyoII distribution and apical constriction in invaginating SG cells were also changed in *arc* mutants providing a mechanistical explanation for geometrical alterations of *arc* mutant SG tubes. Importantly, Arc co-localizes with Crb at the cell-cell junctions of SG placode cells, suggesting that the two proteins could interact directly, likely through one of the Arc PDZ domains and the cytoplasmic PDZ-binding motif found at the C-terminus of Crb. Indeed, we have discovered that the first PDZ domain of Arc is required for its co-localization with Crb. Based on these observations, we propose that Arc functions as a newly discovered player in SG morphogenesis. We hypothesize that Arc modulates Crb dynamics by affecting its membrane stability and/or localization through direct physical interactions. We further propose that the inhibitory actions of Crb on MyoII serve to limit MyoII activity, consequently limiting the number of SG cells that internalize at any given time and ultimately shaping the final dimensions of the mature SG tube.

604F **The role of the Sp/KLF transcription factor Huckebein during tubular organ formation in *Drosophila*.** Jeffrey J Matthew, Vishakha Vishwarkarma, SeYeon ChungBiological Sciences, Louisiana State University, LSU

During morphogenesis, epithelial remodeling commences with the coordinated internalization of the flat epithelium. This process is largely dependent on the presence or absence of key cellular processes such as mitosis. In the *Drosophila* embryonic salivary gland (SG), proper tissue internalization (invagination) is required for the formation of an elongated tubular gland. This process is dependent on the Sp1/KLF transcription factor Huckebein (Hkb), and disruption of Hkb protein function results in a spatial distortion in SG invagination, making cells invaginate at the center rather than at the dorsal/posterior corner of the placode. Aside from a role in directing the invagination start site in the SG, little is known about the potential genetic regulatory mechanisms under control by Hkb during development. Using *hkb* CRISPR/Cas9 knockout fly lines, we reveal the role of Hkb in contributing to cell survival during embryonic SG morphogenesis. A subset of SG cells die in *hkb* mutants during invagination, which leads to a significant reduction of the number of SG cells and the formation of a tiny, round-shaped gland at later stages. Blocking apoptosis in the SG in *hkb* mutants rescues the centralized invagination pattern, restores SG cell numbers, and leads to the formation of properly elongated glands, suggesting that abnormal cell death is the major underlying reason of the *hkb* phenotypes. Using Fly-FUCCI, we also reveal aberrant cell cycle status in *hkb* mutant SG cells compared to wild type. Importantly, reducing key cell cycle genes, such as *CycE* and *Cdk1* in the *hkb* mutant background almost fully rescues the *hkb* mutant phenotypes, suggesting that disrupted cell cycle by the loss of *hkb* results in apoptosis of SG cells and subsequent morphological defects during SG morphogenesis. These cell cycle genes are ChIP-Seq targets of Hkb (ModENCODE project), suggesting a role of Hkb in regulating the transcription of key cell cycle genes. Our data suggest a previously unknown role of Hkb in regulating key cell cycle effector genes to ensure cell survival and proper SG morphogenesis. Overall, our study reveals a key regulatory mechanism of the cell cycle for the proper tubular organ formation during development.

605F **Expanding the ocellar gene regulatory network in *Drosophila melanogaster*** Karly Miller, Claude B Jean-Guillame, Bonnie M Weasner, Justin P KumarBiology, Indiana University at Bloomington

Gene regulatory networks (GRNs) describe the interactions between transcription factors and signaling molecules that direct development of undifferentiated cells towards a specific cell fate. Throughout development, the cooperativity of components from multiple GRNs are required to generate various tissues within a single organism. One such GRN is the retinal determination network (RDN), a set of conserved factors that are responsible for proper eye development. The RDN has been extensively studied in the context of the Drosophila melanogaster compound eye as most flying insects have two compound eyes, and generally three ‘simple eyes’ called ocelli. While the compound eye is used to accurately see an organisms’ environment, ocelli are used for detecting overhead light and assisting flying behaviors. Ocelli and the compound eyes develop from the same larval imaginal disc, the eye-antennal disc (EAD). Two EADs transform during

pupation from thin, sac-like epithelial sheets into the adult eyes, antennae, and surrounding head capsule. Though the RDN has given much insight into compound eye development, an in-depth network for ocellar development has yet to be characterized. The overall goal of this project is to expand the ocellar-GRN (OC-GRN).

I plan to further investigate known RDN genes, as well as new ocellar-specific genes. By using the existing OC-GRN, in addition to unpublished RNAi screen data, I have an excellent starting point to map out ocellar determinants. I performed a GAL4-RNAi screen using the *dve*GAL4 driver and looked at RNAi lines for 158 transcription factors. *Defective proventriculus* (*dve*) is involved in ocellar development and lies at the top of the OC-GRN. The *dve*GAL4 driver is expressed solely in the ocellar region of the eye disc at 115hr, which is when the ocellar field is specified. By expressing RNAi knockdowns in the ocellar-specific region, I can ensure that the compound eye is not affected by my manipulations, as the compound eye and ocelli share many developmental genes. My preliminary data shows that RNAi knockdown of genes in the ocellar region has differing effects. In the screening process I have binned different genes into categories based on mutant ocellar phenotypes: no ocellar region, no ocelli, reduced ocelli, spacing, and fusion. This work not only renews interest in ocelli and adds ocellar-specific genes to the OC-GRN, it further contributes new insights to the field of eye formation.

606F **Ubiquitous and tissue-specific G protein-coupled receptors work together to fine-tune extracellular signal during tubular organ formation** Vishakha Vishwakarma, SeYeon ChungBiological Sciences, Louisiana State University

During epithelial tube formation, cells actively change their shapes and arrangements to make a three-dimensional structure. Rho-dependent actomyosin contractility is crucial to generate cellular forces to drive cell shape changes and rearrangement. Rho signaling is activated by G protein-coupled receptor (GPCR) signaling at the cell surface in both invertebrates and vertebrates. During *Drosophila* SG invagination, the GPCR ligand Folded gastrulation (Fog) activates Rho signaling to drive apical constriction. Two GPCRs, Smog (ubiquitous) and Mist (mesoderm-specific) regulate myosin contractility downstream of Fog in the early *Drosophila* embryo. However, the SG receptor(s) for Fog that translates Fog signal to cytoskeletal reorganization has not yet been identified, and how Fog signaling is regulated in different tissues also not well understood. Using genetic suppression assay and in vitro cell contraction assay, we revealed that Smog transduces Fog signal to regulate Rho kinase (Rok) and myosin accumulation in the apicomedial region of SG cells to control apical constriction during invagination. We also discovered Fog-independent roles of Smog in regulating different pools of myosin and the actin cytoskeketal networks and maintaining epithelial integrity. Since apical constriction is a widespread cell shape change that occurs in different tissues during development, we hypothesize that tissue-specific GPCR(s), such as Mist in the mesoderm, may work alongside ubiquitouly expressed Smog to effectively transduce Fog signal. From our pilot screen, we identified two additional tissue-specific GPCRs that are upregulated in tubular organs and respond to Fog. We are currently testing our hypothesis that tissue-specific GPCRs work with Smog to fine-tune Fog signal for proper apical constriction in each organ.

607F **Optogenetic Techniques for Control of the Bone Morphogenetic Protein (BMP) Pathway in S2 cells** Shelby Morton, Greg ReevesChemical Engineering, Texas A&M University

The dorsal-ventral (DV) axis determination in drosophila is controlled by two major protein gradients, Dorsal and Decapentaplegic (Dpp). Dpp is a homolog of TGF-β and activates serine threonine kinases (STK), punt and thickveins, which dimerize and recruit additional receptors to form a heterotetramer resulting in phosphorylation of Mothers Against Dpp (Mad) allowing for signal transduction. Target gene activation is determined by a Dpp concentration gradient (covering the dorsal 30% of the embryo) and is classified into subsections based on gene activation. These subsections are named Type I, Type II, and Type III genes where Type I genes are expressed at the dorsal midline where Dpp concentration is highest and Type III genes are expressed at about 25% of the DV axis. The Dpp concentration gradient is dynamic during nuclear cycle (nc) 14 because its expression narrows to about 5-6% of the DV axis while Type III genes continue to be expressed. It is not currently known how these genes remain active after dpp signal recession.

Using optogenetic techniques where a light-oxygen voltage (LOV) domain which dimerizes in the presence of blue light is added to both STK receptors of Dpp, the activation of the BMP pathway can be controlled. This precise activation/inactivation of Dpp signaling allows us to elucidate the gene regulatory network responsible for prolonged activation of Type III genes in the absence of Dpp. By exposing embryos or S2 cells transiently to blue light, we expect to mimic the activation of Type III genes. This will allow us to perform RNA-seq and ATAC-seq and determine differentially expressed genes that may control Type III gene regulation.

608F **Investigating the effects of phosphorylation on dSmad2 degradation** Pablo Flota, John Guanzon, Edward

EiversBiological Sciences, California State University Los Angeles

The TGF-β signaling family is divided into two primary pathways; the Activin/TGF-β pathway and Bone Morphogenetic Protein pathway. TGF-β signals have been shown to be important for cell proliferation, differentiation and cell fate determination during embryonic development and tissue homeostasis in adults. Both pathways transmit their intracellular signals via a group of proteins known as receptor Smads (R-Smads). These transcription factors transmit each pathways intracellular signals when phosphorylated in their C-terminal domains by ligand-activated transmembrane receptors, ultimately causing repression or activation of target genes. In a number of animal species, it has been demonstrated that activated R-Smad proteins are degraded by series of cellular events, which include, R-Smad phosphorylation in their central linker domains by proline dependent kinases, followed by ubiquitin ligase binding, and finally polyubquitination and degradation by cellular proteasomes. We and others have shown that disruption of linker phosphorylation sites can stabilize R-Smad proteins and prolong the duration of pathway signals. Our lab is currently investigating the mechanisms leading to dSmad2 degradation. It has been previously demonstrated that in *Drosophila* cultured cells, dSmad2 C-terminal phosphorylation causes its bulk degradation. Here we will present our findings investigating if proteasomal inhibitors or various dSmad2 linker mutants can stabilize activated-dSmad2 proteins. *In vivo*, we find that misexpression of dSmad2 linker mutant transgenes in the developing wing imaginal disc resulted in adult wings that were reduced in size and had significant disruption of wing venation, when compared to controls. In conclusion, our experiments aim to broaden our understanding of how *Drosophila* dSmad2 signals are regulated.

609F **Molecular analysis of Toll receptor function during the polarization of contractile and adhesive proteins during *Drosophila* embryogenesis** Chloe Kuebler[1], Adam Paré[21]BISC, University of Arkansas, [2]University of Arkansas

Elongation of the head-to-tail axis is a conserved process during animal development, and it occurs through remodeling of epithelial sheets via cell intercalation. The process of cell intercalation is driven by the polarization of contractile proteins (e.g., myosin) and adhesive proteins (e.g., Par-3) within the plane of the epithelium. An outstanding question is how planar polarity is established consistently across large groups of cells. In *Drosophila*, three Toll receptors—*Toll-2, Toll-6*, and *Toll-8*—are expressed in a complex pattern of vertical stripes, and planar polarity in triple mutants lacking these three receptors is severely disrupted. However, it is unclear how these three receptors function together to generate uniform polarity across the tissue. Preliminary data suggest that these three receptors can function independently, but it is still not known whether they play distinct roles in specific cells. All Toll family receptors (including vertebrate Toll-like receptors) contain extracellular leucine-rich repeats and a highly conserved intracellular TIR (Toll/Interleukin-1) domain. In addition, *Drosophila* Toll-2, Toll-6, and Toll-8 contain extended C-terminal regions—the functions of which are not well understood. To consistently quantify which receptor regions are required for polarity, we have created a genetic system in which endogenous planar polarity is absent and can be induced in response to an ectopic horizontal stripe of transgene expression. Currently we are performing structure-function analyses of the Toll-2 receptor using this system, and we are characterizing the ability of specific Toll-2 protein regions to induce planar polarity. Narrowing down the protein regions required for proper cell intercalation will help reveal the downstream interactions involved in tissue remodeling during *Drosophila* embryogenesis.

610F **Exploring how basement membrane mechanics evolve over time to control tissue lengthening in the *Drosophila* egg chamber** Victoria A Hoznek, Sally Horne-BadovinacMolecular Genetics & Cell Biology, University of Chicago

Organs are formed, in part, by an imbalance of physical forces that drive changes in tissue shape. Many established models of morphogenesis focus on cell-intrinsic force anisotropies generated by actomyosin contractility, but we are beginning to appreciate that tissues can also be shaped by cell-extrinsic forces from the extracellular matrix (ECM). We are using the *Drosophila* egg chamber, which is surrounded by a basement membrane (BM) ECM and elongates >2-fold along its anterior-posterior (A-P) axis, to study how differential patterning of ECM mechanics can be used to lengthen an organ.

In early egg chamber development, follicular epithelial cells migrate along the BM, causing the egg chamber to rotate and deposit BM proteins that form polarized fibrils in the previously uniform matrix[1]. These fibrils help to create a "molecular corset" along the A-P axis with a softer BM at the poles of the egg chamber and a stiffer BM in the central region[2]. The molecular corset, in turn, channels the expansive growth of the germ cells along the A-P axis to lengthen the tissue. However, loss of fibrils still yields substantial elongation[3], suggesting that undiscovered mechanisms contribute to the mechanical anisotropy of the matrix. Because Collagen IV (Col IV) is the main determinant of BM stiffness, we are

exploring other ways that the Col IV network is stiffened and softened during these stages.

Later in development, egg chamber rotation and BM fibril formation stop, yet tissue growth and BM-dependent elongation continue. Simultaneously, germ cell growth becomes asymmetric as the oocyte at the posterior rapidly takes up yolk proteins[4]. The symmetrical A-P stiffness gradient in the BM transforms into a sharp division between a soft anterior and stiff posterior during these stages[2,5], but it is not known how or why the molecular corset evolves post-rotation. Our preliminary data suggest that a drastic change in BM composition may be responsible for the shift in BM mechanics to accommodate asymmetric growth of the germ cells. We are using both established and novel methods to test this hypothesis.

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611F **Using multiphoton fluorescence lifetime imaging (FLIM) to visualize NADH/FAD endogenous autofluorescence in Drosophila early embryogenesis** Maria Espana, Adam PareUniversity of Arkansas

Tissue remodeling occurs in the context of both normal development and wound healing. By studying tissue remodeling events across a range of organisms—notably convergent extension (CE) during head-to-tail axis elongation—researchers have identified conserved contractile and adhesive proteins that alter cell morphology. In addition, the processes that drive CE share striking similarities at the molecular level with epithelial wound healing, and many view epithelial regeneration as a reactivation of normal developmental mechanisms. Notably, wound healing is also dependent on mitochondrial dynamics (i.e., fusion and fission) and the production of reactive oxygen species. It is therefore compelling to think that changes in cellular metabolism or mitochondrial signaling may be necessary to drive the dynamic cellular processes of CE, although this has not been demonstrated *in vivo*. One of the premier models for studying CE is the *Drosophila* embryo, because 1) cell movements are easy to visualize, 2) CE occurs rapidly over the course of 30 minutes, and 3) tissue remodeling occurs in the absence of cell division (which complicates analysis in other systems). To address whether there are changes in cellular metabolism over the course of CE in living embryos, we are using non-destructive multiphoton microscopy and fluorescence lifetime imaging (FLIM) to visualize the endogenous autofluorescence of mitochondrial cofactors before, during, and after CE. We are also using fractal image analysis to probe mitochondrial network architecture to ask whether mitochondrial dynamics are correlated with CE. These experiments will reveal the role of cellular bioenergetics during tissue remodeling in the context of normal development, and may contribute to our understanding of the cellular basis of wound healing and CE-based birth defects, such as spina bifida.

612S **The Puratrophin-1-like RhoGEF regulates epithelial tube formation during *Drosophila* embryonic development** Thao P Le[1], Zahra Naeini[2], SeYeon Chung[21]Biological Sciences, Louisiana State University, [2]Louisiana State University

Drosophila embryonic salivary gland (SG) and trachea are two powerful models to study how three-dimensional tubes are formed from flat polarized epithelial sheets. Among >20 Rho-specific guanine nucleotide exchange factor (RhoGEF) genes that exist in Drosophila, two ubiquitously expressed RhoGEFs have been shown to be involved in epithelial morphogenesis: RhoGEF2 and Dp114RhoGEF/Cyst. We show the role of another RhoGEF, Puratrophin-1-like (Pura), in epithelila tube formation and germband retraction during embryogenesis. *In situ* hybridization data shows that *pura* is highly upregulated in the developing SG and trachea throughout morphogenesis with basal expression levels in other epithelial cells. Using CRISPR/Cas9, we generated a null mutant line of *pura* that deletes the DH and PH domains of the protein essential for the RhoGEF function. Homozygous *pura* mutant embryos are viable and fertile, but ~70% of *pura* mutant embryos show delayed germ band retraction defects. Embryos with more severe morphological defects are often slightly twisted, with reduced levels of the key apical protein Crumbs (Crb) in patched regions of the epidermis. *pura* mutant embryos also show apical constriction defects during SG invagination and disorganized medioapical myosin web structures compared to control. *pura* mutant embryos also show a range of defects in several tracheal branches, including gaps in the dorsal trunk, overly convoluted dorsal trunk, and mismigrated dorsal branches. Defects in the SG and trachea also corelate with mislocalized/discontinuous Crb signals in the cells. Based on the yeast-two-hybrid data that showed Rac1 as a potential binding partner of Pura, we are currently testing our hypothesis that Pura activates both Rho1 and Rac1 activities to regulate epithelial morphogenesis. Different isoforms of Pura show different subcellular localization, either enriched in the apical domain or uniformly distributed in the cytoplasm, suggesting that different isoforms of Pura may activate Rho signaling in different subcellular domains. Overall, our data show that *pura* plays critical role in epithelial morphogenesis and epithelial tube formation, potentially by activating Rho1 and Rac1 activities

at different subcellular domains in different developmental contexts.

613S **Investigating the role of Dachsous intracellular domain and binding partners in wing size and planar cell polarity using CRISPR.** Alex J Earl[1], Heya Zhao[2], Alexey Veraksa[2], Helen McNeill[11]Developmental biology, Washington University in St. Louis, [2]University of Massachusetts Boston

Fat (Ft) and Dachsous (Ds) are massive cadherins, 560kDa and 350kDa. Ft regulates tissue growth, planar cell polarity (PCP), morphogenesis, and mitochondrial metabolism through the heterophilic binding of Ds. This binding is thought to alter both Ft and Ds signaling. Although much work has been done on the Ft intracellular domain (ICD), little is known about the Ds ICD. The Ds-ICD has regions of high conservation, but their in vivo function is unknown. We used CRISPR to delete a 20aa region in the conserved motif 2(CM2). Interestingly, loss of the CM 2 results in flies with smaller wings. In contrast, deletions of CM in Ft-ICD typically show an overgrowth phenotype. To identify proteins that mediate Ds signaling, we conducted proteomic analysis using endogenous Ds that was GFP tagged co-immunoprecipitated TTC28 as a top interactor. The zebrafish ortholog of TTC28 interacts with Zebrafish *dchs1b* via the Dchs1 conserved motif. Ttc28 limits microtubule turnover and is a conserved regulator of cell division and microtubules which controls embryonic cleavages. To determine the biological relevance of the TTC28-Ds interaction in *Drosophila*, we generated TTC28 null flies via CRISPR. I will present my work exploring why the loss of the Ds conserved domain inhibits wing growth, describing the effects of loss of TTC28, and determine if TTC28 binding to Ds is important for growth signaling.

614S **Involvement of Btz in Grk / EGFR Signaling during Drosophila Oogenesis** Alexander J. Mathewson, Lillian J. Markley, Scott B. FergusonBiology, State University of NY at Fredonia

During oogenesis localized expression of Gurken (Grk) establishes the dorsal-ventral (D/V) axis of the oocyte. Mutations in *spn-B* lead to insufficient translation of *grk* during oogenesis and a failure to correctly establish D/V asymmetry. We have identified two novel EMS-generated alleles of *barentsz* (*btz*) that suppress the ventralized phenotype of eggs laid by *spn-B^{BU}* females. CRISPR/Cas9 induced mutations of *btz* recapitulate this finding. Btz is a core component of the Exon Junction Complex (EJC), a protein complex that forms at exon-exon junctions of mature mRNAs. The EJC is involved in nuclear export, localization, and translational enhancement of mRNA. Here we will provide insights into the role of Btz in the control of *grk* translation.

615S **Downstream transcriptional targets of *Drosophila* Egf receptor signaling play roles in eggshell morphogenesis** Lisa Kadlec[1], Zachary Walter[2], Corrine Brown[3], Alexis Morgan[4], John Nawrocki[5], Michael Warkala[61]Biology and Earth System Sciences, Wilkes University, [2]Microbiology and Immunology, Thomas Jefferson University, [3]Catalent Pharma, [4]Geisinger, [5]Internal Medicine, Christiana Care, [6]Molecular Biology, Genetics, and Cancer, Rutgers University

The *Drosophila melanogaster* ovary is a remarkable model system for understanding both the patterning and morphogenesis of tissues. During oogenesis, both anterior-posterior and dorsal-ventral axes are established in the egg and key morphological features, including the dorsal respiratory appendages, are formed. The respiratory appendages are one of several Drosophila morphological features, including the trachea and salivary glands, that have been used to study the process of tubulogenesis. Epidermal growth factor receptor (EGFR) signaling has for some time been known to play a key role in the patterning of eggshell features along both major body axes during oogenesis. The EGF receptor is activated by a gradient of the ovarian EGFR ligand gurken, which induces the expression of genes canonically involved in this patterning. 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 analysis has revealed loss of function phenotypes ranging from improper tubulogenesis of the dorsal respiratory appendages to a catastrophic loss of chorion integrity and subsequent severe reduction in fertility, and has thus identified multiple genes activated by EGFR signaling that have roles in the morphogenesis, rather than the patterning, of eggshell features.

616S **Spatiotemporal dynamics of the protein Cactus in *Drosophila melanogaster* development** Allison E Schloop[1,2], Sharva Hiremath[3], Cranos Williams[3], Gregory Reeves[21]Genetics, North Carolina State University, [2]Chemical Engineering, Texas A&M University, [3]Electrical and Computer Engineering, North Carolina State University

Development of an organism is dependent upon proper regulation of gene expression. Initiation of gene expression often relies on long-range signals referred to as morphogens; these morphogens form concentration gradients that aid in specific activation of genes responsible for proper body patterning. In *Drosophila*, the transcription factor Dorsal (Dl), a homolog of vertebrate NF-kappaB, acts as a morphogen to pattern the dorsal-ventral (DV) axis in the early embryo. Cact, a homolog of vertebrate IkappaB, is initially bound to Dl, sequestering it to the cytoplasm. Toll signaling on the ventral side of the embryo degrades Cact and allows Dl to enter the nucleus to regulate target gene expression. Ultimately, Dl activity specifies multiple gene expression domains along the DV axis to initialize the embryo fatemap. While this information has been long established, previous work in the Reeves lab suggests Cact also enters the nucleus; mathematical models of the Dl gradient fail to accurately predict the normal range of the gradient without nuclear Cact. However, direct, *in vivo* visualization of Cact spatiotemporal dynamics, including its localization to the nuclei, has been difficult to gather.

Previously, imaging Cact in live embryos was complicated by rapid protein turnover, preventing fluorescent protein fusions from fully maturing. To overcome this challenge, we tagged Cact with the recently developed “LlamaTag” (LT), a genetically encodable nanobody from llamas that dynamically binds to GFP *in vivo* (Bothma et al., 2018). We show that Cact-LT/GFP has a predominantly cytoplasmic pattern but is also present in the nucleus with a nuclear-to-cytoplasmic fluorescence ratio of 1/3. Fluorescence recovery after photobleaching (FRAP) of the nucleus shows a recovery time scale of 5-10 min. This contrasts with control flies (GFP only) which have no cytoplasmic pattern and have a rapid FRAP time scale (30 s), and strongly suggests that Cact-LT is present in the nucleus. We then used raster image correlation spectroscopy (RICS) and found that roughly 50% of nuclear GFP is bound to Cact-LT. Collectively, our results show there is a significant pool of Cact in the nucleus, which suggests that Cact may regulate Dl in the nucleus.

617S **A Single Transcription Factor Determines Distinct Visual System Fates** Claude B Jean-Guillaume, Justin P KumarBiology, Indiana University

The specification of the Drosophila eye is controlled by two Pax6 orthologs, Eyeless (Ey) and Twin of eyeless (Toy). These are thought to split the duties of ancestral Pax6 by regulating distinct target genes. However, since eye development can proceed normally in animals that are null for each individual gene, Ey and Toy are likely activating the same set of target genes. Ey is a stronger transcriptional activator than Toy, thus each Pax6 gene is predicted to activate identical targets but at distinct levels. Since several tissue express either one or both Pax6 genes, we asked if differences in the transcriptional strength of Ey and Toy specify unique fates. We show that specification of the ocelli requires a level of Pax6 activity that is distinct from the compound eye. The activation of common targets at distinct levels is an important, yet underappreciated, mechanism by which diverse cell fates are specified

618S **The Serine-like Protease *masquerade* (*mas*) Plays an Important Role in Tracheal Tube Formation** Victoria Kurdyumov, Afshan IsmatBiology, 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 and has been shown to be involved in proper axonal guidance and somatic muscle attachment, perhaps acting as a competitive antagonist to other serine proteases in the extracellular matrix. Our preliminary data suggests that *mas* also plays an important role in tracheal tube formation. In the absence of *mas*, we found that a significant percentage of mas mutant embryos had major defects in the trachea. Specifically, individual tracheal metameres had missing dorsal trunk or dorsal branches while the rest of the trachea formed normally. We are currently in the process of marking the entire membranes of all tracheal cells to see if there is any change to cellular protrusions or membrane degradation. We are also in the process of generating untagged and GFP-tagged over-expression constructs of *mas* to be able to examine what happens in the trachea with too much Mas present. Overall, this work will be instrumental to our understanding of tracheal tube formation.

619S **Evaluating the effect of molecular interaction between *Tsh* & *CtBP* on *Drosophila* eye development** Harley Hines[1], Raven Newton[1], Surya Jyoti Banerjee[21]Biology, Arkansas Tech University, [2]Biological Sciences, Arkansas Tech University

Distinct combinations of conserved transcription factors regulate division of the eye precursor cells, followed by induction of photoreceptor cell specification in the *Drosophila, (*the fruit flies) larval eye precursor tissue called the eye

disc. The eye disc is a sac made of two epithelial cell layers. Both layers grow by cell division until the second instar larval stage. During the third instar larval life, a morphogenetic furrow (MF) made of indented cell layers originates at the posterior margin of the eye disc and propagates towards the anterior side of the eye disc. The cells anterior to the MF are in the proliferating stage, and cells posterior to it start to differentiate as photoreceptors. The differentiated retinal cells form the units of the compound adult eyes in *Drosophila*. Previous studies have shown that Teashirt (Tsh), a zinc finger transcription factor, promotes cell division anterior to the MF. The C-terminal Binding Protein (CtBP), a conserved transcriptional co-repressor, was shown to limit cell division in the eye disc. Interestingly, our immunoprecipitation assays revealed that Tsh and CtBP molecules interact *in vitro* and *in vivo* in the eye discs. Thus, our present research goal is to identify, weather the molecular interaction is associated with the eye development pathways in the *Drosophila.* We have developed fly strains with over-expression of *tsh* & *CtBP* in the dividing cells anterior to the MF of the eye disc. As a result, we found that there is no or tiny adult eyes in the flies with *tsh* over-expression, and subtle smaller adult eye developed in the flies with *CtBP* over-expression. Next, we plan to make a double mutant fly by over-expressing both *tsh* & *CtBP* to evaluate the effect of their interaction on eye phenotype. The result will help to identify the processes of eye development regulated by the Tsh and CtBP together.

620S **Scraps, an anilin, and Nebbish, a kinesin, are integral components of a Fox transcription factor-regulated subnetwork that mediates specific 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[1,2,31]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

Fox transcription factors mediate multiple cardiogenic processes in both mammals and *Drosophila*. The *Drosophila* Fox genes *jumeau* (*jumu*) and *Checkpoint suppressor homologue* (*CHES-1-like*) mediate three categories of cardiac progenitor cell division—asymmetric, symmetric, and cell division at an earlier stage*. jumu* also regulates the expression of the kinesin Nebbish (Neb) and the activity of the kinase Polo to mediate symmetric and earlier cardiac progenitor cell divisions in a *CHES-1-like*-independent process. By comparing expression profiles, we identified *scraps* (*scra*), an anilin-encoding gene*,* that like *neb*, is also transcriptionally activated by *jumu*, but not by *CHES-1-like*. Phenotypic analysis of mutations show that *scra*, like *neb*, is required for only two of the three categories of *jumu*-regulated cardiac progenitor cell division—symmetric and cell division at an earlier stage. Synergistic genetic interactions between *scra*, *neb*, *jumu*, and *polo*, and the absence of such synergistic interactions between either *scra* and *CHES-1-like* or *neb* and *CHES-1-like*, demonstrate that *scra* and *neb* are integral components of a *jumu*-regulated subnetwork mediating a specific subset of cardiac progenitor cell divisions. Preliminary data from our phenotypic analysis of other exclusively *jumu*-regulated genes suggests that the kinesin Pavarotti, the citron kinase Sticky, and the Rho GTPase Tumbleweed may be other components of this subnetwork. Using genetic interaction and rescue assays, we are attempting to position *neb* and *scra* topologically relative to each other and these other potential subnetwork components.

621S ***trithorax* is essential for cardiac *Hox* gene expression and anterior-posterior patterning of the *Drosophila melanogaster* embryonic dorsal vessel** Adam J Farmer, Shaad M Ahmad, Kristopher R. SchwabBiology, Indiana State University

The *Drosophila melanogaster* embryonic heart (dorsal vessel) is a linear contractile tube composed of myoepithelial cardiac cells (CCs) with a wide lumen heart proper region that propels hemolymph anteriorly through a narrower aorta region to the anterior embryo. The dorsal vessel is further segmented into repeated 'hemisegments' containing Seven-up (Svp) CCs and Tinman (Tin) CCs. The colinear expression of *Antennapedia* (*Antp*) and the *Bithorax Complex* (*Bx-C*) *Hox* genes along the dorsal vessel length patterns the aorta and heart proper region, as well as the hemisegments. While *Hox* genes have well established roles in dorsal vessel patterning, little is known about their cardiac specific regulation. Within the developing embryo, *Hox* gene expression is positively regulated by *trithorax group* genes. We have identified the COMPASS-like H3K4 methyltransferase gene, *trithorax*, as an essential regulator of colinear cardiac *Hox* gene expression and anterior-posterior dorsal vessel patterning. *trx* inactivation in *Drosophila* causes a remarkable homeotic transformation of the posterior heart proper segment into an aorta-like fate as shown by the loss of heart proper markers and cardiac Abdominal-A (Abd-A), the *Bx-C Hox* gene that confers heart proper specification. Furthermore, cardiac expression of Antp, Ultrabithorax (Ubx), and Abdominal-B (Abd-B) is also dysregulated within the *trx* mutant. Antp and Abd-B are lost within the aorta and posterior terminus, respectively. As expected from the loss of Antp, the *trx* null dorsal vessel shows a loss of a Svp-CC in the anterior most hemisegment. Additionally, excess Tin-CC proliferation at the posterior terminus of the dorsal vessel was observed as expected from the loss of Abd-B. Ubx was maintained

at low levels throughout the dorsal vessel thereby maintaining hemisegment patterning within the aorta and heart proper. Together, these data suggest the COMPASS-like histone methyltransferase *trx* is essential for cardiac *Hox* gene expression and anterior-posterior dorsal vessel patterning. Most notably, *trx* inactivation leads to a profound homeotic transformation of the heart proper into an aorta-like fate due to the loss of Abd-A.

622S **The adult *Drosophila* salivary gland exhibits an unusual mode of cell division** Caitlin van Ree, Harshaa Chandrasekaran, Nicole Dominado, Nicole A Siddall, Gary R HimeAnatomy and Physiology, University of Melbourne

Larval salivary glands of *Drosophila* are well known to exhibit polytene cells formed via endoreplication and have been long used to study this process. In contrast, little is known of the development of adult *Drosophila* salivary glands except that they consist of a single layer, tubular epithelium that originates from a population of diploid cells found as an imaginal ring near larval salivary gland ducts. We have shown that the adult salivary glands contain three distinct epithelial domains, two of which are comprised of cuboidal epithelial cells and one of squamous epithelial cells. These cell types develop during the pupal period and after eclosion secretory cells develop extensive apical membrane invaginations. The junctional polarity of the epithelial cells exhibits an unusual change soon after eclosion as E-cadherin localisation migrates from a position apical to the septate junction to a more basal position. We have found that polyploid adult epithelial cells are not mitotic, yet total cell numbers increase within 2 days of eclosion. By using genetic tools designed for the MARCM lineage tracing technique we have shown that the polyploid cells lose chromosomes during the division period and appear to be using amitosis as a mechanism to increase cell number. Amitosis is a form of cell division undertaken by polyploid cells that does not require establishment of a mitotic spindle and results in chromosome loss. Amitosis is also observed in polyploid giant cancer cells within many epithelial tumour types and permits tumours to regenerate diploid tumour cells and also to evade chemotherapeutic drugs. We have identified the first evidence for amitosis involvement in primary formation of a tissue and the adult *Drosophila* salivary gland will serve as a model for genetic analysis of this mode of division.

623S **Analyzing the role of Approximated-mediated palmitoylation in the Fat/Dachsous signaling pathway** Xing Wang[1], Alex Murphy[2], Jose Cruz-Arzon[3], Seth S Blair[41]Beijing Key Laboratory of Biodiversity and Organic Farming, China Agricultural University, [2]Genetics, University of Wisconsin, [3]Integrative Biology, University of Wisconsin, [4]Integrative Biology, Univ Wisconsin

The giant protocadherins Fat and Dachsous (Ds) form a heterophilic, bidirectional signaling pair that regulates proliferation via the growth-inhibiting Hippo pathway, and planar cell polarity (PCP) both through and independently of the Fz/Vang PCP pathway. Fat, Ds and the effectors of the Hippo and PCP pathways are concentrated in the subapical cortex of epithelial cells, and the intracellular domain (ICD) of Fat has strong effects on the subapical levels of two critical proteins. The first is the scaffolding myosin Dachs; Dachs inhibits Warts, the final effector kinase in the Hippo pathway, and regulates Sple in the core PCP pathway. The second is the FERM scaffolding protein Expanded (Ex), which stimulates Warts and inhibits Yorkie activity. We previously established a link from the Fat ICD to Dachs and Ex levels and localization via the DHHC palmitoyltransferase Approximated (App) and the SH3 domain protein Dlish. We hypothesized that App-mediated palmitoylation of Dlish recruits it to apical cell cortex where it binds and stabilizes Dachs and destabilizes Ex. To test this model, we have identified the palmitoylated cysteines in Dlish and mutated these to test function. We show that mutant Dlish that cannot be palmitoylated loses its subapical localization and its growth-regulating activity in vivo, creating a dominant negative form of Dlish that still binds to but mislocalizes Dachs to the cytoplasm. The activity of the mutant Dlish can, however, be rescued by adding back an exogenous lipidation site to the protein, suggesting that the only critical role of the mutated cysteines is to act as palmitoylation sites. We have also identified domains in Dlish critical for binding App and show these are similarly critical for Dlish activity. We will present preliminary data on sites in App and other DHHC family members that regulate target specificity, and evidence for the involvement of additional proteins in the regulation of DHHC activity.

624S **Quantitative 3D mechanical model of embryonic epithelium based on *in vivo* mechanical measurements** Mohamad Ibrahim Cheikh, Konstantin DoubrovinskiBioPhysics, UT Southwestern Medical Center

In order to understand morphogenesis, it is necessary to know the material properties or forces shaping the living tissue. In spite of this need, very few *in vivo* measurements are currently available. Using the early *Drosophila* embryo as a model, we describe a novel cantilever-based technique which allows for the simultaneous quantification of applied force and tissue displacement in a living embryo. By analyzing data from a series of experiments in which embryonic epithelium is subjected to developmentally relevant perturbations, we conclude that the response to applied force is

adiabatic and is dominated by elastic forces and geometric constraints, or system size effects. Crucially, computational modeling of the experimental data indicated that the apical surface of the epithelium must be softer than the basal surface, a result which we confirmed experimentally. More specifically, apical domains form a continuous surface behaving as a stiff elastic sheet, whereas the basal cellular edges comprise a floppy hexagonal network since cells are open on the basal side during the cellularization stage when measurements are performed. We find that the more rigid basal network is largely responsible for the force opposing the motion of the cantilever during the pulling, whereas the apical domains are mainly responsible for the recoil observed after the removal of the force. In this way, strikingly, different cellular domains contribute differently to the different phases of tissue deformation. Further, we used the combination of experimental data and comprehensive computational model to estimate the elastic modulus of the apical surface and set a lower bound on the elastic modulus of the basal surface. More generally, our investigations revealed important general features that we believe should be more widely addressed when quantitatively modeling tissue mechanics in any system. Specifically, different compartments of the same cell can have very different mechanical properties; when they do, they can contribute differently to different mechanical stimuli and cannot be merely averaged together. Additionally, tissue geometry can play a substantial role in mechanical response, and cannot be neglected.

625S **Chitinase-Like Proteins work through a novel signaling pathway to regulate tube formation** Luana Paleologu[1], Claudia Espinoza[2], Celeste Berg[11]Genome Sciences, University of Washington, [2]University of California San Diego

Tubes are the foundation for many organs in all multicellular organisms, yet the molecular signaling pathways that control tube formation are poorly understood. The Berg lab studies tube formation in *D. melanogaster* egg chambers, which form tubes that fill with chorion protein to become dorsal appendages, eggshell structures that bring oxygen into the embryo. The Berg lab discovered that a class of secreted proteins known as chitinase-like proteins are upregulated in a mutant with deformed dorsal appendages, and over-expression of a single member of this gene family causes aberrant cell migration and open tubes. Chitinase-like proteins are conserved across the animal kingdom, with six paralogs present in *D. melanogaster* known as Idgf1-6. The human orthologues are elevated in inflammatory disorders and cancers, but their mechanism of action is unknown. To identify potential signaling components, a previous genetic screen in the Berg lab identified *eIF3e* as a strong enhancer of the *Idgf3*-overexpression phenotype. eIF3e is a translation initiation factor that regulates translation of specific mRNAs, including *Mical*. Mical oxidizes F-actin to facilitate fiber disassembly, acting as a key mediator of cytoskeleton remodeling. Changes in epithelial sheet architecture, including tube formation, rely on changes to the actin cytoskeleton, making Mical an attractive link between eIF3e and Idgfs. We hypothesize that Idgf3 inhibits Mical by reducing eIF3e activity, which in turn stabilizes actin fibers to prevent remodeling during tubulogenesis. To explore the link between Idgfs and eIF3e, we are investigating the finding that Tor signaling controls eIF3-component expression. Tor inhibits Thor, which inhibits the eIF4F complex, which is responsible for translating components of the eIF3 machinery. We hypothesize that Idgf signaling inhibits Tor, ultimately inhibiting the translation of eIF3e and Mical. Uncovering the signaling pathway of chitinase-like proteins will further our understanding of cell migration and tube formation.

626S **Lipid modified FGF programs cytoneme-mediated polarized FGF signaling and tissue organization** Sougata Roy[1], Lijuan Du[2], Alex Sohr[21]University of Maryland, College Park, [2]University of Maryland

During morphogenesis, cells spatiotemporally coordinate with each other by communicating with secreted signals. Cells transmit signals target-specifically through cytoneme contacts. We investigated how cytoneme contacts form and why signals are released only though the cytoneme contact sites. In *Drosophila*, FGF produced in wing disc cells regulates the development of the disc-associated air-sac-primordium (ASP). ASP and wing-disc cells extend cytonemes containing FGFR and FGF, respectively on their surfaces. These FGF sending and receiving cytonemes reciprocally guide each other to establish contacts and exchange FGF directly at the contact sites. Further investigation revealed that the contact-dependent reciprocal guidance and signal exchange are controlled by a lipid-modification of the FGF. FGF is GPI-anchored to the source cell surface. This lipid-modification inhibits random FGF secretion, but facilitate contact-dependent target-specific FGF release. We show that disc and ASP cells dynamically extend cytonemes and recognize each other by Cell-Adhesion-Molecule/CAM-like FGF – FGFR interactions at the cytoneme contacts. Contact-mediated FGF-FGFR binding induces reciprocal responses in ASP and source cells which polarize their cytonemes toward each other and mutually stabilize the signaling contact sites. FGFR-bound FGF is then released from the source GPI-anchor that enables the FGFR-bound FGF to move to the ASP cell body via ASP cytonemes. Thus, lipid-modification enable the FGF to orchestrate bidirectional FGF-FGFR signaling, which, in turn, controls the target-specific origin of cytoneme contacts and contact-dependent self-regulated FGF release.

627S **Differential bazooka levels regulated by a novel Drosophila protein, Moat, define morphogenetic boundaries** Lingkun Gu[1], Rolin F Sauceda[2], Mo Weng[31]School of life sciences, UNLV, [2]Stanford University, [3]UNLV

Although morphogenetic boundaries are defined by the expression of patterning genes, they are executed through the differential activity patterns of general cellular machineries such as cell adhesions and molecular motors. Here we report an uncharacterized Drosophila gene, moat, that plays a role in defining the differential patterns of cell adhesion and modulating the boundaries of morphogenetic events. Moat is dynamically expressed in many future morphogenetic structures in early embryos. Loss of moat leads to increased junctional localization of polarity protein Bazooka, resulting in increased adherens junctions (AJs). This appears to weaken the differential levels of Baz and junctions and disrupts the invagination of mesoderm, stomodeal anterior mid gut (AMG) and dorsal folds. In wild type embryos, shortly before mesoderm undergoes epithelial folding, Baz localization and AJs are downregulated in both mesoderm and AMG cells due to the expression of Snail. Such a downregulation allows the subsequent strengthening of AJs in response to contractile actomyosin only in the mesoderm but not the stomodeal AMG. In moat mutants, Baz and AJs remain at high levels in both tissues despite the normal expression of Snail. Interestingly, in the mutants, the mesoderm epithelial folding is not restricted to mesoderm but is extended to include the stomodeal AMG. However, the expressions of patterning genes such as Giant, Huckebein and Snail are normal. Strikingly, RNAi knockdown of Baz is sufficient to rescue the ectopic extension of the epithelial fold, suggesting the elevated Baz and AJs underlie the changed boundary. In addition, within the mesoderm, the elevated junctional Baz and AJs appear to disrupt a ventral-dorsal gradient of AJs, which is formed in response to a gradient of contractile actomyosin following the Snail-dependent Baz and junction downregulation. The loss of such a junction gradient is associated with an uncoordinated and delayed folding of mesoderm. Lastly, moat mutants also show defects in dorsal fold formation, which is driven by differential positioning of Baz and AJs. In moat mutants, we observe frequent loss of the anterior fold as well as the formation of ectopic folds, suggesting overly stable junctional Baz may interfere with the differential remodeling of Baz position. In summary, moat appears to function downstream of fate determinants to modulate the tissue boundaries of cell adhesion strength and morphogenetic structures.

628S **The multimodal action of *Gαq* in coordinating growth and homeostasis in the *Drosophila melanogaster* wing imaginal disc** Maria F. Unger[1], Vijay F. Velagala[1], Dharsan K. Soundarrajan[1], Nilay Kumar[1], David Gazzo[1], Jun Li[2], Jeremiah Zartman[11]Biomolecular Engineering, University of Notre Dame, [2]Department of Applied and Computational Mathematics and Statistics, University of Notre Dame

G proteins mediate cell responses to ligands such as growth factors, hormones, and neurotransmitters and play key roles in organ development. A diverse range of ligands binds to G protein-coupled receptors (GPCRs), which activate the dissociation of the G-protein complex into the *Gα* and *Gβγ* subunits. For example, dissociated G protein *Gαq* subunit regulates a broad range of downstream signals mediated by key second messengers, including calcium (Ca^{2+}) ions. Dysregulation of G- proteins or Ca^{2+} signaling is implicated in the etiology of many diseases, including cancer, diabetes, and poor immune response. However, the downstream effectors of G protein activity in developmental regulatory networks are still poorly understood.

The *Drosophila melanogaster* wing disc is a powerful model system to explore the features of GPCR signaling in relation to epithelial morphogenesis. The Gal4/UAS binary system was used to perturb Gαq levels in the wing disc, followed by phenotypic analysis and immunohistochemistry to characterize the effect of *Gαq* perturbations. Next-gen RNA sequencing was conducted on wing discs with *Gαq* perturbations to identify the downstream effectors affected by the disruption of *Gαq* homeostasis.

Here, we characterized how the G protein subunit *Gαq* tunes the size and shape of the wing through multiple tissue-specific perturbation studies. Overexpression (OE) of *Gαq* is sufficient to promote global Ca^{2+} waves in the wing disc. *Gαq*RNAi and *GαqOE* cause reduction in the final size of the *Drosophila* wing and also delay in pupariation. The reduced wing size phenotype is further enhanced when downregulating downstream components of the core Ca^{2+} signaling toolkit, suggesting that downstream Ca^{2+} signaling partially rescues the growth phenotype. In contrast, *Gαq*OE-mediated pupariation delay is rescued by inhibition of IP3R, a key regulator of Ca2+ signaling. This suggests that Gαq regulates developmental phenotypes through both Ca^{2+}-dependent and Ca^{2+}-independent mechanisms. We demonstrate that disruption of Gαq homeostasis regulates nuclear hormone receptors, JAK/STAT pathway, and immune response genes. In addition, disruption of Gαq homeostasis increases expression levels of relaxin-like peptide Dilp8, a key regulator of growth inhibition and pupariation timing.

Gαq activity regulates cell size and metamorphosis and acts through nuclear hormone receptors, JAK/STAT pathway, and immune response genes. Disruption to *Gαq* homeostasis in the peripheral wing disc organ delays organism development through inhibition of ecdysone pathway. Overall, *Gαq* homeostasis is necessary for coordinated growth, apoptosis, and developmental timing, thereby achieving an optimal organ size. In sum, our results suggest that Gαq signaling mediates key modules of organ size regulation and epithelial homeostasis through the dual action of Ca^{2+}-dependent and independent mechanisms.

629S **A cell adhesion gradient contributes to gastrulation morphogenesis in *Drosophila*** Nat Clarke[1], Adam Martin[21]Biology, Massachusetts Institute of Technology, [2]Massachusetts Institute of Technology

The morphogenetic movements that give rise to the structures of the adult body are powered by motility and shape change at the cellular level, but these events must be coordinated at larger spatial scales to enable proper morphogenesis. For example, adjacent cell populations often exhibit distinct and complementary behaviors, such as a population of cells stretching or moving to allow a neighboring tissue to constrict or fold. How such coordination arises, and whether it is in response to embryonic patterning cues or mechanical cues, is still unclear in many systems.

Here, we report evidence of a tissue-wide cell adhesion pattern within the ectoderm of the *Drosophila* embryo that supports the tissue movements of the mesoderm during gastrulation. Using a quantitative imaging approach, we found a graded pattern of components of the adherens junction (AJ) is present in the ectoderm prior to the onset of gastrulation: levels of DE-Cadherin, Armadillo (beta-catenin), and p120-catenin are enriched at sub-apical cell junctions in the dorsal and ventro-lateral ectoderm and reduced in the lateral ectoderm. This AJ pattern corresponds closely to the pattern of EGFR-Ras-Raf-MAPK pathway signaling activity in the early blastoderm embryo. To test whether this pathway is upstream of AJ patterning, we used genetic and pharmacological perturbations to block EGFR activity and saw that inhibition of EGFR produces gastrulation defects by inhibiting cell displacement and deformation. We are currently testing a model in which variable levels EGFR activation tune levels of cell-cell adhesion to modulate regional differences in mechanical properties within the ectoderm to enable tissue movement; we are testing this model through a combination of in vivo experimentation and computational modeling.

630S **Investigating how force regulates mitotic entry timing during Drosophila gastrulation** Mingmar Sherpa, Adam C Martin, Jaclyn CamugliaBiology, Massachusetts Institute of Technology

Cell division and cell contractility and shape changes contribute to tissue morphogenesis. Cell division is tightly regulated by biochemical factors that influence the cell cycle. Past work in our lab showed how contractility of mesoderm cells influences cell division orientation during Drosophila gastrulation. Whether contractility can influence the timing of mitotic divisions and how this coordinates contractility and cell division during gastrulation is unknown. During Drosophila gastrulation, cell division is regulated by mitotic entry of cells in stereotypic regions across the embryo, termed mitotic domains. Here, we examine the role of forces in the timing of mitotic entry in the mitotic domains. To do this, we have disrupted adherens junctions to halt force transmission generated during mesoderm invagination. In addition, we examine the timing of mitotic entry in *snail* mutants where the mesoderm doesn't undergo contractility or invaginate. Preliminary evidence suggests a role for mesoderm invagination in mitotic domain timing. We are also investigating possible mechanisms by which force may regulate mitotic entry in the embryo.

631S **Optogenetic reconstitution of apicobasal shortening in early embryonic epithelia** Andrew D Countryman[1], Maya S Puri[2], Marisol Herrera-Perez[2], Caroline A Doherty[3], Stanislav Y Shvartsman[3], Karen E Kasza[21]Biomedical Engineering, Columbia University, [2]Mechanical Engineering, Columbia University, [3]Molecular Biology, Princeton University

Epithelial tissues in developing embryos undergo extensive remodeling to create the diverse forms of adult animals. While the nascent field of synthetic morphology has made significant advances in replicating a small number of morphogenetic processes, the reconstitution of a wider range of tissue shape changes for science and engineering applications remains a challenge. Optogenetic manipulation of Rho signaling has proven to be a promising avenue to achieve spatial, temporal, and quantitative control over actomyosin-generated contractile forces and tissue folding processes in epithelia. Here, we use CRISPR/Cas9 gene editing in *Drosophila melanogaster* to generate a novel optogenetic tool via the endogenous tagging of RhoGEF2 with components of the iLID/SspB optogenetic system. This endogenous tagging approach is designed to produce flies with normal levels and patterns of RhoGEF2. The resulting flies are homozygous-viable and are generally healthier than flies overexpressing a comparable transgenic tool. Blue light activation of the tool in the embryonic ectoderm recruits RhoGEF2 to the cell membrane. Notably, we find that spatially localized activation can induce apicobasal shortening of cells within the targeted area under certain illumination

conditions, a shape change known to occur in several *in vivo* morphogenetic processes. This shortening is often accompanied by apical cell area expansion and membrane blebbing, and leads to an overall narrowing of the tissue along the apical-basal axis. Local activation of the tool in the converging and extending germband is sufficient to drive cell shortening in the intercalating tissue, demonstrating the ability to override the existing morphogenetic program and to explore how out-of-plane deformations in a tissue interact with in-plane remodeling processes. Varying the size, geometry, and activation intensity of the targeted contractile region reveals how tissue-scale tensile force transmission and mechanical coupling between cells influence cell shortening. In conclusion, this work characterizes a novel optogenetic approach to reconstitute and study apicobasal shortening and lays a foundation for the use of calibrated control of contractility to precisely specify tissue shapes and direct tissue movements.

632S **Expression patterns of lexA and split-GAL4 drivers in enteroendocrine cells of Drosophila melanogaster**
Ellen Popodi, Jessica Holsopple, Kevin CookBiology, Indiana University

The Drosophila melanogaster midgut is a valuable model tissue for characterizing and manipulating cells involved in intestinal endocrine signaling. As in other organisms, the Drosophila intestine contains secretory cells called enteroendocrine cells which release peptide hormones to induce systemic physiological effects in response to changing intestinal conditions. Enteroendocrine cells secrete many combinations of peptide hormones and genetic tools are needed to identify, target, and manipulate discrete subpopulations. The Chemoconnectome Project has developed GAL4 and lexA drivers to report expression of peptide hormone genes, but the expression of these drivers has not been characterized thoroughly in the midgut. The split-GAL4 system also holds promise for experimentally identifying and manipulating enteroendocrine cell subpopulations. Here, we characterize Chemoconnectome Project driver expression in the adult midgut. We then use these driver expression patterns to characterize enteroendocrine cell subpopulations defined by coexpression with split-GAL4 driver pairs. This work provides a simple framework for evaluating the experimental potential of other split-GAL4 pairs in the future.

633S **Interaction between the dorsal selector gene *defective proventriculus* (*dve*) and Decapentaplegic (Dpp) signaling pathway during *Drosophila* development eye.** Neha Gogia[1], Anuradha Venkatakrishnan Chimata[1], Anjali Sangeeth[1], Katie Perry[1], Madhuri Kango-Singh[1,2,3,4], Amit Singh[1,2,3,4,51]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

During organogenesis, axial patterning is required to establish the Antero-Posterior (AP), Dorso-Ventral (DV), and Proximo-Distal (PD) axes for proper organ development. The DV axis is the first lineage restriction event during eye development. These processes like patterning and development are carefully co-ordinated by various transcription factors, morphogens and signalling pathways. Any errors in this process result in developmental defects, genetic birth defects, and patterning defects in the organ. Here we wanted to study the interaction between a previously identified dorsal selector gene *defective proventriculus* (*dve*, an ortholog of SATB1), a K-50 homeodomain containing transcription factor and Dpp morphogen. Decapentaplegic (Dpp)/Bone Morphogenetic Protein (BMP) signaling pathway is highly conserved in humans and forms morphogen gradient in the developing eye to initiate retinal differentiation and establish the anterior-posterior axis of the *Drosophila* eye imaginal disc. We hypothesize that *dve* could interact with Dpp signaling and an optimum level of interaction between *dve* and Dpp signaling is essential for the proper development of *Drosophila* eye. Our results suggest Dve might interact in an antagonistic manner with Dpp pathway to regulate growth. We will address this hypothesis using gain-of-function and loss-of-function approaches. Here we present how the *dve* patterning gene interacts with the Dpp signaling pathway to determine retinal vs head cuticle fate.

634S **Studying the role of Hedgehog signaling pathway in Dorso-Ventral patterning** Soumya Bajpai[1], Anuradha Venkatakrishnan Chimata[2], Amit Singh[3,4,5,6,71]Department of Biology, University of Dayton, [2]Department Of Biology, University of Dayton, [3]Department Of Biology, University Of Dayton, [4]Premedical Program, University of Dayton, [5]Center for Tissue Regeneration and Engineering at Dayton (TREND), University of Dayton, [6]Integrative Science and Engineering (ISE), University of Dayton, [7]Center for Genomic Advocacy (TCGA), University Of Dayton

During organogenesis, three-dimentional organs arise from a monolayer of cells and this requires axial patterning to establish Dorsal-Ventral (DV), Anterior-Posterior (AP) and Proximal-Distal (PD) axes. Among them, the DV axis is the first to form during eye development. Morphogens have also been associated with a multitude of developmental processes, including organ patterning and the control of organ size. The Hedgehog (Hh) family of molecules play an

important role 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 wanted to understand how Hh signaling interacts with a previously identified dorsal selector gene, *defective proventriculus (dve).* Dve is a K-50 homeodomain containing transcription factor, a *Drosophila* ortholog of human SATB1. We know that loss-of-function of *dve* results in dorsal eye enlargement while gain-of-function results in complete eye suppression. We wanted to understand how this transcription factor can regulate Hh signaling pathway that is crucial for patterning, growth and eye development. Here, we present our results from modulating Hh signaling pathway in *dve* expression domain and how it affects eye development.

635V **Midline and Morphogens: Coordination of Furrow Dynamics in the Eye Disc with Cell Fate Specification and Tissue Patterning** Sandra Leal[1], Sandra Leal[2], DaMarrion .White[3], Aliyah Edwards[31]Biology, Harris-Stowe State University, [2]Biology, HARRIS STOWE STATE COLL, [3]HARRIS STOWE STATE COLL

We are seeking to uncover mechanisms regulating cellular pre-pattern formation using the developing eye imaginal disc of *Drosophila* as a model tissue. We hypothesize that a putative population of *pre*-sensory organ precursor (P-SOP) cells of larval eye discs mark a geometric pattern that corresponds to the pattern of interommatidial bristle placement in the adult eye. P-SOP cells are predicted to exist in a quiescent state until pupal development when they become competent to process Notch-Delta signaling and assume neuronal fates. Subsequently, each neuronal SOP cell gives rise to a lineage of daughter cells that produces the terminal cell types that comprise an interommatidial bristle complex. We reported previously that the *midline* (*mid*) transcription factor gene can be genetically placed within the Notch-Delta signaling pathway regulating the neuronal cell-fate specification and viability of SOP cells (Das et al., 2013; Chen et al., 2015). Presently, we predict that *mid* may play a much earlier role in selecting and positioning P-SOP cells into stereotypic locations occupied later by interommatidial bristles. Preliminary studies show that the long-range morphogen, Decapentaplegic (Dpp), is required to inhibit Mid expression within the pre-neuronal zone of the eye disc were such a population of P-SOP cells likely reside. Morphogens are secreted signaling molecules that regulate tissue patterning in a concentration-dependent manner. The pre-neuronal zone flanks the anterior margin of the morphogenetic furrow (MF), a contractile wave of differentiating tissue giving rise to neuronal cells. In addition, long-range Dpp signaling is required to maintain the expression of Groucho (Gro), a repressor of Mid activity. Currently, we are investigating whether the short-range morphogen, Hedgehog (Hh), in concert with the long-range Dpp signal cooperate to regulate Mid and Gro expression within the pre-neuronal zone as a prelude to support a Turing model of pre-SOP cell selection and spatial positioning within this zone that precedes the arrival of the MF and is self-perpetuating along the anterior to posterior axis.

636V **How The Thanos Requirement Leads To An End Game On Wing Fate During Ectopic Eye Formation** Alison Smith, Justin KumarBiology, Indiana University Bloomington

During multicellular development, cells are in a pluripotent state though due to various cell signaling pathways and morphogen gradients cells begin to determine. When a cell does not adopt or retain the correct fate during development it can lead to various developmental anomalies. One way this happens is by transdetermination. Transdetermination is when a determined cell switches its fate to that of another tissue type without going through dedifferentiation. This process of transdetermination can be studied in *Drosophila melanogaster* via ectopic eye formation through the misexpression of the retinal determination gene *eyeless* (*ey*). For an ectopic eye to develop via transdetermination the cell must abandon its original fate to adopt a retinal fate. Using the UAS-GAL4 misexpression system I have shown that there is a critical spatial expression, molecular combination, developmental time, and magnitude level to which *ey* misexpression must occur for ectopic eye formation. Also known as the Thanos requirement for ectopic eye formation, because if the criteria are met it leads to an end game on wing date during ectopic eye formation, but if it is not met the cells can return to their original fate. My results also indicate that this transdetermination occurs via a biphasic cell state, which is a completely new finding in work involving transdetermination. By understanding the requirements of transdetermination we can gain a better understanding of the process of cell fate determination, and why only some cells within an imaginal disc can undergo transdetermination while others cannot.

637V **Extradenticle expression in the *Drosophila Melanogaster* eye regulates ectopic patterning on the ventral margin of the eye-antennal imaginal disc** Jasmine Warren[1], Justin Kumar[21]Indiana University Bloomington, [2]Biology, Indiana University Bloomington

Many kinds of eye diseases, including those that result in blindness originate from genetic mutations of key genes

involved in eye morphogenesis. *Extradenticle (exd),* is a TALE homeobox family transcription factor that is used in a variety of molecular processes such as embryogenesis and development of the central nervous system. However, it's role in patterning the *Drosophila* eye has not been completely understood. The compound eye of *Drosophila* is composed of 800-unit eyes, called ommatidia, which are arranged in a stereotyped hexagonal array. This organization, down to the directional angle of the ommatidia, is key to proper vison in adult flies. This specific cell organization is achieved by a wave of cell differentiation called a morphogenic furrow. This furrow arises from a single point on the most posterior end of the eye-antennal imaginal disc and migrates anteriorly in a single wave of differentiation. This patterning event involves several signaling pathways to properly pattern the undifferentiated cells that composed the eye field. My preliminary findings have shown a role for *exd* in retina patterning, which revolves around the regulation of cell differentiation on the ventral side of the *Drosophila* eye. During a genetic screen I found that reductions in levels of Exd leads to the inappropriate release of a second wave of differentiation from the ventral margin of the eye field. This was accomplished by utilizing RNAi and a unique driver, c311-GAL4, that drives expression in a specific tissue layer of the imaginal disc known as the peripodial epithelium. This ectopic patterning results in a completely disorganized retina. A possible cause for the disorganized retina is that *wingless (wg)* expression is lost on the ventral margin when *exd* is knockdown. It has been well documented that *wg* signaling in the eye acts as a repressor to other key patterning genes. Additionally, I conducted a genetic screen to identify potential binding partners of *exd* that functional in patterning the *Drosophila* eye. The results of my screen showed that the knockdown of *hth* in the peripodial epithelium phenocopies the loss of *exd*. This data shows that *hth* and exd maybe functioning as co-factors to properly spatially restrict the initiation and progression of cell differentiation in the *Drosophila* eye disc.

638V **Morphodynamics of Early Drosophila Embryogenesis** Noah P Mitchell[1], Matthew F Lefebvre[1], Vishank Jain-Sharma[1], Nikolas Claussen[1], Marion K Raich[2], Hannah J Gustafson[3], Andreas R Bausch[4], Sebastian J Streichan[51]Physics, University of California, Santa Barbara, [2]Physics, Technical University of Munich, [3]Biomolecular Science and Engineering, University of California, Santa Barbara, [4]Technical University of Munich, [5]University of California, Santa Barbara

While many aspects of *Drosophila* embryogenesis are well understood as distinct processes, a quantitative characterization of global flow remains elusive. We have generated a large (> 100 embryos) database of live-imaged movies of *Drosophila* gastrulation which allows for the generation of an ensemble representation of global tissue flow. Quantitative analysis of surface tissue flow demonstrates that while individual cells are transported hundreds of microns during gastrulation, the instantaneous flow pattern characterizing how cells move from one time point to the next does not change. Additionally, the time-averaged flows of individual embryos are nearly identical to the ensemble-averaged flow, which strongly suggests that patterns of tissue flow are driven by egg geometry. When we perturb this system by characterizing the dynamics of gastrulation at different temperatures, we observe that the maximum velocity and time duration of germ band extension flow scale with temperature. As temperature increases, the maximum velocity of flow is increased, and the time duration of flow is correspondingly reduced, in such a way that the total spatial tissue extension remains the same. Our analysis therefore suggests that geometric motifs are encoded during the process of *Drosophila* morphogenesis.

639V **Integrins and the Notch pathway control epithelial cell proliferation and fate specification in the *Drosophila* ovary** Lourdes Rincón-Ortega, Andrea Valencia-Expósito, Anna Kabanova, María Dolores Martín-Bermudo, Acaimo González-ReyesCABD, Spanish National Research Council (CSIC)

Integrins are essential proteins that connect the cell with the extracellular matrix (ECM). They interact with signaling pathways to regulate epithelial morphogenesis and homeostasis. Using the follicular epithelium of the *Drosophila* ovary as a model system, we show that integrins control the number of polar and stalk cells by regulating the proliferation of epithelial cell precursors in the germarium. These phenotypes are similar to those found in ovarioles with decreased levels of Laminins in their ECM, pointing to a role for the integrin-mediated cell-ECM interaction(s) in the control of epithelial division and fate acquisition. In addition, we demonstrate that the loss of integrin function in germarial stages produces an overactivation of the Notch pathway. Because decreasing Notch pathway activity rescues the proliferation and cell fate specification phenotypes caused by the lack of integrins, we propose that integrin binding to the ECM is required for proper Notch signaling in the germarium, particularly during the proliferation of polar cell and stalk cell precursors.

640V **The Hippo-like Ccm3 signaling pathway regulates tube morphogenesis via Rab11.** Amin S GhabrialPathology and Cell Biology, Columbia University

The fly ortholog of *Cerebral Cavernous Malformation 3* (*CCM3*), a human vascular disease gene, regulates tracheal tubulogenesis. Ccm3 binds to and potentiates the activity of Germinal Center Kinase III (GckIII) family members. In *Drosophila*, *wheezy* encodes the sole GckIII. Previously, we showed that mutations in *wheezy/GckIII* caused cystic tube dilations in the seamless tubes of tracheal terminal cells. We have now generated a *Ccm3* knockout allele and find that, in mosaic animals, it recapitulates the *wheezy* terminal cell defects; however, there is a strong maternal component to *Ccm3* expression, causing partially penetrant defects. When maternal *Ccm3* is eliminated and zygotic clones are induced, *Ccm3* mutant terminal cells show a fully penetrant tube dilation defect. We also show that maternal/zygotic *Ccm3* null embryos have additional tube defects, with the multicellular dorsal trunk tube showing increased length as well as tube constrictions and dilations. In prior studies, we showed that GckIII phosphorylates and activates the *Drosophila* NDR kinase, Tricornered. We showed that mutations in *tricornered* cause terminal cell tube defects similar to those caused by loss of GckIII function. We now show that mutations in *Ccm3*, *Mo25*, and *GckIII* cause multiple wing hair defects like those characteristic of *trc* mutations. Taken together, our studies identify a signaling cassette that operates in at least two tissues and includes: Mo25 acting with Ccm3 and GckIII, to activate Trc, which partners with Furry (Fry) and Monopolar spindle (Mps) one binding 2 (Mob2). How the pathway regulates tube morphogenesis remains the key unanswered question in the field, but we now share data suggesting that excess recycling of Rab11 vesicles to the apical domain contributes to the defect, and that loss of Rab11 activity is sufficient to suppress tube dilation.

641V **TOR signalling regulates epithelial cell shape transition in *Drosophila* oogenesis** Mohit Prasad[1], Sudipta Halder[2], Gaurab Ghosh[21]Indian Institute of Science Education and Research Kolkata, [2]biological Sciences, IISER Kolkata

Epithelial morphogenesis plays an important role in form generation, organ development and maintenance of adult tissues in the metazoans. Given its wide implication, aberrant morphogenesis is linked to severe developmental defects and in few instances also associated with tumorigenesis. Employing the model of *Drosophila* oogenesis, we have examined the role of evolutionary conserved Target of Rapamycin (TOR) kinase pathway, a known regulator of cell growth and size in mediating shape transition of cuboidal cell to squamous epithelial fate. Utilizing genetic tools, immunohistochemistry and live cell imaging, we demonstrate that TOR signaling is active and required for epithelial morphogenesis during *Drosophila* oogenesis. Further, loss of function analyses indicates that non canonical TOR signaling functions through PAR-1 to mediate the removal of lateral cell adhesion molecule, Fasciclin2, to allow proper squamous cell morphogenesis. In addition, we demonstrate the effect of TOR through PAR-1 on cell shape transition is mediated via modulation of endocytosis. Over all, our data gives novel insight into the how TOR signaling mediates cell shape transition during epithelial morphogenesis in the metazoans.

642V **Specialized cells that sense tissue mechanics to regulate morphogenesis** Hui-Yu Ku[1], Leigh Harris[1], David Bilder[21]UC-Berkeley, [2]Univ California, Berkeley

Shaping of developing organs requires dynamic regulation of force and resistance to achieve precise outcomes, but how organs monitor tissue mechanical properties is poorly understood. We show that in the developing Drosophila follicle (egg chamber), a single pair of cells at the anterior pole performs such monitoring to drive organ shaping. These polar cells secrete a Matrix Metalloproteinase (MMP) which specifies the appropriate degree of tissue elongation, rather than hyper- or hypo-elongated organs. MMP production is negatively regulated by basement membrane (BM) mechanical properties, which are sensed through focal adhesion signaling and autonomous contractile activity; MMP then reciprocally regulates BM remodeling, particularly at the anterior region. Remarkably, changing BM mechanics at remote locations alone is sufficient to induce a remodeling response in polar cells. We propose that this small group of cells senses both local and distant stiffness cues to produce factors that pattern the organ's BM mechanics, ensuring proper tissue shape and reproductive success.

643V **Glypican-based mechanisms of extracellular Wnt distribution** Indrayani Waghmare, Andrea Page-McCawVanderbilt University

Wnts are evolutionarily conserved secreted ligands that form extracellular gradients to direct several cellular behaviors at short- and long-ranges. The distribution of extracellular Wnts is primarily dependent on cell-surface localization of glypicans, which distribute Wnts by continual binding and release in the plane of the tissue. Because glypicans play an important role in modulating extracellular Wnt availability, their cell-surface levels are likely regulated by other factors. In the *Drosophila* germarium, a tissue where oogenesis initiates, the glypican Dally-like protein (Dlp) promotes long-range extracellular Wg, the *Drosophila* ortholog of mammalian Wnt1, distribution from Wg-producing cap cells to Wg-responsive follicle stem cells inducing their proliferation, required for egg development. Both knockdown of

dlp or tethering Wg to cap cell membrane disrupts follicle stem cell proliferation. In genetic experiments, Matrix Metalloproteinase 2 (Mmp2) inhibits Dlp's long-range Wg distribution to restrict Wg signaling in follicle stem cells. Thus, Mmp2 acts as a molecular break on Dlp's long-range function. Mechanistically, we discovered that in S2R+ insect cells, Mmp2 cleaves Dlp on the cell surface and destabilizes it. Additionally, based on the known crystal structure of Dlp, cleavage of Dlp by Mmp2 likely induces a conformational change where the cleaved pieces remain held together by a di-sulfide bond, and the cleaved protein is internalized and degraded. Interestingly, cleaved Dlp sequesters more Wg than intact Dlp. Based on these and our previous observations, we propose a model wherein intact Dlp on the cell surface promotes long-range Wg distribution. In contrast, cleavage by Mmp2 destabilizes Dlp, and cleaved Dlp sequesters more ligand, removing the Dlp-Wnt complex from the cell surface, resulting in attenuation of ligand distribution and function. Overall, this study identifies the molecular basis of protease-mediated inhibition of cell-surface glypican, which modulates ligand distribution and function.

644V **Mechanical inputs and Rho1 GTPase signaling regulate medioapical actomyosin network turnover during eye epithelial morphogenesis** Christian Rosa[1], Victor Hatini[21]Tufts University, [2]CMDB, Tufts Universirty

The developing fly retina is a model for understanding how force-generating cytoskeletal networks control epithelial morphogenesis. During epithelial remodeling, cell contacts and the apical cell area repeatedly contract and expand. A junctional actomyosin network that cyclically assembles and disassembles has been shown to control contact length dynamics. Additionally, a dynamic medioapical actomyosin network has been identified, but its function is not understood. The Rho1 RhoGTPase controls the assembly of contractile actomyosin network by activating the Formin Diaphanous (Dia), which assembles linear actin filaments, and non-muscle Myosin II (MyoII), which contracts these filaments. We hypothesized that Rho1 dynamics and actomyosin turnover dynamically control actomyosin contractility and the rebalancing of forces in the epithelium during tissue remodeling. High-resolution live imaging revealed a medioapical actomyosin ring composed of nodes linked by filaments that attract each other and fuse during apical cell area contraction. The network then dissembles, flows, and fuses with the junctional network during apical cell area relaxation. Laser ablation of the medioapical network resulted in a rapid non-isometric relaxation of the apical cell area, indicating that the network contracts the apical cell area asymmetrically. Overexpressing Rho1 accelerated medioapical actomyosin turnover, increased the amplitude of actomyosin accumulation and cell area fluctuations. Medioapical actomyosin network assembly and cell contraction initially led to network disassembly and apical area expansion of adjacent cells. Subsequently, the expanding cell assembled a medioapical actomyosin network that contracted the apical cell area causing adjacent cells to undergo inversely coordinated cycles of expansion and contraction. Overexpressing a constitutively active myosin light chain kinase ($MLCK^{CA}$) or a constitutively active Dia (Dia^{CA}) to disrupt actomyosin turnover led to defects in epithelial remodeling. $MLCK^{CA}$ accelerated the medioapical actomyosin dynamics, while Dia^{CA} inhibited medioapical ring formation and non-isometric cell area contraction. Therefore, it is likely that the primary factor influencing medioapical actomyosin dynamics is MyoII activation patterns. We identified RhoGAP71E and RhoGEF2 as regulators of Rho1-mediated medioapical actomyosin dynamics. While RhoGAP71E inhibited actomyosin ring assembly, RhoGEF2 accelerated medioapical actomyosin dynamics. Overall, our results indicate that Rho1-dependent MyoII activation and inactivation regulate force balance during epithelial morphogenesis. Mechanical inputs from neighboring cells regulate Rho1 function to control actomyosin network assembly and disassembly. RhoGEF2 and RhoGAP71E regulate Rho1 to influence the frequency and amplitude of medioapical actomyosin assembly.

645T **Microtubule polymerase XMAP215/Mini spindles and cytoplasmic dynein are required for the oocyte determination in *Drosophila*** Wen Lu, Margot Lakonishok, Vladimir I GelfandCell and Developmental Biology, Northwestern University

In many species, only one oocyte is specified among a group of interconnected germline sister cells. In *Drosophila melanogaster*, 16-cell interconnected cells form a germline cyst, where one cell becomes the oocyte, while the rest become nurse cells that provide the oocyte with mRNAs, proteins, and organelles through intercellular cytoplasmic bridges via microtubule-based transport. In this study, we find that a microtubule polymerase Mini spindles (Msps), the *Drosophila* homolog of XMAP215, is essential for defining and maintaining of the oocyte fate determination. Knockdown of *msps* blocks the oocyte growth and causes gradual loss of oocyte determinants. *msps* knockdown abolishes microtubule polymerization in the oocyte and results in the absence of microtubules growing from the oocyte to the nurse cells. We demonstrated that cytoplasmic dynein, the main microtubule minus-end directed motor, is required for *msps* mRNA transport to the oocyte, and thus accumulation of translated Msps protein in the oocyte. The dynein-dependent concentration of Msps causes more microtubule plus-ends to grow from the oocyte to nurse cells, further enhancing dynein-dependent nurse cell-to-oocyte transport. Thus, the dynein-Msps duo creates a positive feedback

loop that transforms a slight stochastic difference in microtubule polarity among sister cells into a clear oocyte fate determination.

646T **Bridging the gap between mitochondrial fission and cytokinesis during Drosophila sperm development**
Catherine Q.F. Zhang[1,2], Julie A. Brill[1,21]Molecular Genetics, University of Toronto, [2]Cell Biology, The Hospital for Sick Children

Cytokinesis is a fundamental process that allows for the faithful segregation of nuclear and cytoplasmic contents into daughter cells. Defects in cytokinesis are associated with aneuploidy and can lead to cancer in humans. Thus, obtaining an understanding of which components are required for faithful cytokinesis is crucial. Using the powerful model of Drosophila spermatogenesis, cytokinesis can be easily monitored in the large dividing spermatocytes and resulting spermatids. We previously identified the gene *fwd* to be essential for meiotic cytokinesis. Fwd is a lipid kinase that localizes to the Golgi and adds a phosphate group to phosphatidylinositol to produce PI4P. Our previous results suggested that Fwd was important for generating PI4P for post-Golgi vesicles that are trafficked to the cleavage furrow to support the increase in surface area during cytokinesis. However, new research has uncovered a role for Fwd in promoting mitochondrial division in fly neuronal and muscle cells, opening the door to novel mechanisms behind the previously observed cytokinesis defects in *fwd* mutants. During early sperm development, mitochondria undergo periods of fusion followed by fission prior to meiotic cytokinesis. Thus, it is possible that the loss of Fwd and mitochondrial fission would result in a hyper-fused mitochondrial network that could sterically block cytokinesis from proceeding. To determine whether this occurs, I am quantifying mitochondrial morphologies before and during spermatocyte meiotic division in WT and *fwd* mutants using fluorescent mitochondrial markers and dyes. I will also assess the phenotypes of double mutants of *fwd* and mitochondrial fusion/fission factor genes to determine epistatic relationships and place *fwd* in the fusion or fission pathways. Lastly, to uncover other potential roles of Fwd, I will perform structure-function analysis of the Fwd protein to assess if interactors of the mammalian ortholog, PI4KIIIB, are conserved in flies. Overall, determining the role of Fwd in mitochondrial division will allow for more insight into the regulation of cytokinesis, as well as mitochondrial diseases.

647T **The LIM protein Smallish regulates actomyosin contractility during epithelial morphogenesis in *Drosophila***
Patrizia Kroll, Andreas WodarzAnatomy, University Hospital Cologne

The cortical actomyosin network generates the mechanical force that drives highly dynamic processes occurring during epithelial morphogenesis, such as cell division, cell rearrangements or cell shape changes. Proper apical-basal and planar cell polarity are a fundamental requirements to regulate the spatiotemporal behavior of the actomyosin network during morphogenesis. Cell polarity is determined by the asymmetric distribution of proteins. In *Drosophila*, Bazooka/Par3 (Baz) is one of the key polarity regulators. By protein interaction studies, the LIM domain protein Smallish (Smash) was identified as Baz binding partner.

Mutation of *smash* interferes with planar polarity since it was shown to influence the planar polarized localization of several actomyosin-associated proteins. Besides its involvement in regulating polarity, Smash affects actomyosin contractility, because $smash^{\Delta 35m/z}$ null allele mutants show reduced membrane tension, whereas overexpression of Smash leads to apical constriction in epithelial cells. In $smash^{\Delta 35m/z}$ null mutants, many embryos show strong epithelial morphogenetic defects, likely due to misregulation of the actomyosin network. Although we have several lines of evidence demonstrating that Smash is functioning in a large multi-protein complex to control membrane tension via the actomyosin network, the precise mechanism of how Smash provides its function remains to be elucidated.

To obtain a deeper insight into the molecular working mechanism of Smash, different methods were combined. Smash interaction partners were identified by a proximity labelling technique. The results of these *in vivo* experiments were complemented by *in vitro* interaction studies. The biological relevance of these interactions was investigated by localization studies to test whether Smash influences the subcellular localization of the identified binding partners and vice versa. To better understand the function of Smash, a detailed phenotypic analysis of the *smash* null mutant embryos was conducted. In addition, a GFP knock-in into the Smash locus via CRISPR/Cas9 was performed. The newly generated fly strain serves as a useful tool to investigate the dynamics of GFP:Smash during epithelial morphogenesis *in vivo*.

648T **An Intestinal G Protein-Coupled Receptor modulates enteroendocrine peptide secretion and lipid homeostasis in *Drosophila melanogaster.*** Daniela Barraza[1], Bat E Jugder[2], Rita Wang[3], Lang Liang[4], Paula I Watnick[21]Department of Microbiology, Harvard Medical School, [2]Division of Infectious Diseases, Boston Children's Hospital, [3]Department of Immunology, Harvard Medical School, [4]Harvard School of Dentistry

The intestinal enteroendocrine cell (EEC) senses stimuli through various receptors, including G Protein-Coupled Receptors (GPCRs). Upon activation, EEC GPCRs regulate secretion of enteroendocrine peptides (EEPs) that control important physiological processes including satiety, intestinal contractions, and systemic metabolism. The *Drosophila melanogaster* EEP Tachykinin (Tk) regulates intestinal lipid homeostasis by repressing lipid synthesis in enterocytes and knockdown of *Tk* in EECs (Tk>*Tk*^{RNAi}) results in enterocyte lipid droplet accumulation. Our lab found that *Tk* transcription is regulated through EEC import of microbiota-derived acetate. Interestingly, we recently discovered that mutation of an EEC-specific GPCR, which we temporarily called GPRx, phenocopies EEC-specific knockdown of *Tk*. Based on this and other observations, we hypothesized that Tk is regulated both at the level of transcription and release and that GPRx is the GPCR that regulates release of Tk from EECs. To test this hypothesis, we knocked down *GPRx* in Tk-expressing EECs (Tk>*GPRx*^{RNAi}) and used fluorescence microscopy to quantify intestinal lipids and the number of cells containing detectable Tk peptide. Consistent with our hypothesis, we observed that accumulation of lipids in the intestines of Tk>*GPRx*^{RNAi} flies was not accompanied by a change in the number of Tk-expressing EECs. Additionally, knockdown of an essential exocytosis factor, *Bruchpilot (brp)*, in Tk-expressing EECs also resulted in intestinal lipid droplet accumulation. RNA sequencing analysis of the intestines of Tk>*GPRx*^{RNAi} flies showed differential expression of vesicle trafficking, secretion, and synapse formation genes, suggesting feedback between release and transcription of EEPs. Our data support a model in which intestinal GPRx regulates exocytosis of Tk from EECs.

649T **Multiple roles for the actin mesh in oocytes?** Hannah M Bailey[1], Margot E Quinlan[2,3]1Chemistry and Biochemistry, University of California Los Angeles, [2]Chemistry and Biochemistry, UC Los Angeles, [3]Molecular Biology Institute, University of California Los Angeles

The process of egg development, oogenesis, is highly conserved and crucial for producing offspring. *Drosophila melanogaster* has long served as a model system to understand aspects of egg development, including stem cell and germ cell development, meiosis, cell migration, intercellular signaling, and mRNA localization. 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 and Cappuccino (Spir and Capu). Concurrently, the expression levels of Spir and Capu decrease dramatically at the onset of late oogenesis and removal of the actin mesh. Loss of the actin mesh permits the initiation of fast streaming, thereby mixing cytoplasmic contents and transport of late polarity factors to the posterior. Whether the actin mesh in *Drosophila* oocytes has roles that are distinct from restricting fast cytoplasmic streaming is unknown. Analogous actin meshes, built by Spir and Capu, have been characterized in different systems, such as the mouse oocyte and melanocyte. In these instances, Spir and Capu-built meshworks are critical for long distant transport of cargo. However, the mechanisms appear to differ in these two cell types. I have endogenously tagged Spir and Capu with fluorescent proteins using genome editing. Using these tools, I will determine the localization and expression timing of these nucleators throughout oogenesis, without complications of expression level and temporal control due to the commonly used drivers of the bipartite GAL4/UAS system. Preliminary data shows both are present on punctae that are enriched at the cortex. I will determine when and where they colocalize and study their dynamics to ascertain actin filament orientation and overall mesh organization. These data will contribute to our understanding of the role of the actin mesh during *Drosophila* oogenesis.

650T **An InR-Vps34/PI3P-Kinesin-2 axis accelerates the anterograde axonal transport of a subset of Rab4-associated vesicles in *Drosophila*** Kamaldeep Singh, Asmita Sarkar, Semanti Das, Krishanu RayDepartment of Biological Sciences, Tata Institute of Fundamental Research

Rab4 GTPase organizes endosomal sorting essential for maintaining the balance between recycling and degradative pathways. Elevated Rab4 levels in the CNS have been associated with synaptic atrophy and neurodegeneration in *Drosophila* and humans. Insulin signaling, which activates Rab4-associated membrane trafficking in adipocytes, regulates synaptic plasticity in the CNS. In addition, a reduction in insulin signaling can ameliorate symptoms associated with neurodegeneration and aging. However, the underlying mechanism(s) remain unclear. Using *in vivo* time-lapse imaging of *Drosophila* larvae, we show that insulin signaling in neurons increases the anterograde speed of a subset of Rab4 vesicles through a novel InR-Vps34/PI3P-Kinesin-2 axis, which elevates the overall anterograde flux of these vesicles in the segmental-nerve axons. Further, we find that synaptic influx of Rab4 in CNS of *Drosophila* larvae is periodically altered during development, and is inversely correlated with the synaptic density in this region. Together, these observations delineate a potentially unique role of insulin signaling in stimulating the movement of a subset of Rab4-associated vesicles through the ESCRT complex in axons and its impact on synaptic stability.

651T **Defining the Role of Adipose Triglyceride Lipase in *Drosophila* Border Cell Migration** Israel WipfUniversity

of Iowa

Lipid droplets (LDs) are dynamic cellular organelles responsible for mediating key steps in lipid metabolism and signaling. LD dysregulation and accumulation is thought to promote cancer invasion and metastasis, but the precise role of LDs in cell migration is unknown. One possibility is that LDs regulate cell migration by modulating cytoskeletal dynamics. Recently, we have identified the conserved LD lipase, Adipose Triglyceride Lipase (ATGL), as a novel regulator of actin remodeling during *Drosophila* oogenesis. Homozygous loss of ATGL results in severe actin defects, impacting both actin bundle formation and cortical actin integrity. Genetic interaction assays indicate that ATGL's control over actin remodeling is dependent on prostaglandin (PG) signaling, with ATGL acting upstream of Pxt, the cyclooxygenase-like enzyme in Drosophila responsible for PG synthesis. Intriguingly, prior work in our lab has demonstrated that PGs are critical orchestrators of cell migration during *Drosophila* oogenesis. This, together with ATGL's PG-dependent control over actin remodeling, leads us to hypothesize that LDs and ATGL might also contribute to PG-dependent cell migration. To test this hypothesis, we use border cell migration during Stage 9 of Drosophila oogenesis. We analyzed the migration of border cells in wildtype vs *ATGL* null flies and found that loss of ATGL results in both delayed migration and failed delamination. These findings suggest a novel role for both ATGL and LDs in facilitating on-time border cell migration, which is necessary to produce fertilization-competent oocytes. Future work will aim to determine whether ATGL and Pxt work in a linear pathway to control on-time border cell migration, as well as to identify other downstream targets of ATGL in migration (*e.g.,* JAK-STAT pathway components which are necessary for border cell specification and delamination). Since *Drosophila* border cell migration is an *in vivo* model of collective, invasive cell migration, these findings are relevant beyond development in processes such as wound healing and cancer invasion or metastasis. Finally, given that LD dysregulation is implicated in human diseases such as diabetes, obesity, and cancer, new insights into the diverse functions of LDs and their associated proteins are likely to lead to improved understanding of disease etiology and new therapeutic targets.

652T **Control of Crag's localization and activity in the polarized deposition of basement membrane proteins in epithelial cells.** Hemin Shah[1], Alex Hoover[1], Trudi Schüpbach[2], Olivier Devergne[11]Biological Sciences, Northern Illinois University, [2]Princeton University

Epithelial cells play critical roles in the development and maintenance of an organism, and the establishment and maintenance of apical-basolateral polarity (ABP) are essential to their function and integrity. ABP is established, maintained, and tightly regulated via intracellular trafficking and environmental cues, such as those provided by the basement membrane (BM). The BM is a specialized sheet of extracellular matrix accumulating and underlying epithelial cells on their basal side. Despite the important roles of the BM in the architecture and functions of epithelial cells, little is known about the mechanism ensuring the exclusive basal restriction of the BM components. To study BM deposition, we use the follicular epithelium (FE) of the *Drosophila* ovary as a model system. The GEF/RabGTPase complex Crag/Rab10 is a key regulator of the biological pathway specifically dedicated to the basal restriction of BM components. However, the exact mechanism responsible for Crag's polarized intracellular localization and its activity in BM polarity remains yet to be elucidated. In FE, Crag assumes a polarized localization and accumulates apically and laterally through yet unknown mechanisms. Importantly, specific localization of the GEF Crag is critical for the localized activation of Rab10. Thus, to understand how Crag controls the polarized deposition of BM proteins, it is important to determine how Crag's activity and localization are controlled. Crag is a multidomain protein containing the DENN domains at the N-terminus responsible for its GEF activity, a Calmodulin Binding Site (CBS) domain responsible for its calmodulin-binding activity, and a conserved C-terminus domain. To determine the domain(s) necessary for Crag's localization and activity, we performed a structure-function analysis. Our data suggest that the CBS domain, but not the DENN domains, is important for the localization of Crag to the apical and lateral domains, suggesting a role of calmodulin in the subcellular localization of Crag. The DENN domains, however, are required, but not sufficient, to control the basal restriction of the BM components to the basal side of the epithelial cells, suggesting that the proper localization of Crag is required for its control of the polarized secretion of basement membrane proteins. Altogether, our data shed a light on the regulation of the activity and localization of Crag, a key component of the biological pathway that controls BM polarity.

653T **Bicaudal-D cargo binding differentially regulates Dynein activity** Frederick C Baker, Phylicia A Allen, Hannah Neiswender, Rajalakshmi Veeranan Karmegam, Gradyon B GonsalvezDepartment of Cellular Biology and Anatomy, Augusta University

The development of the *Drosophila* egg chamber requires the minus end-directed microtubule motor, Dynein. Unlike other motors, only one Dynein variant is involved in cytoplasmic cargo transport. To gain motility and transport cargo,

Dynein must interact with activating cargo adaptors. The most well-studied adaptor of Dynein is Bicaudal-D (BicD). Mutations in mammalian BicD are associated with a subtype of Spinal Muscular Atrophy, a degenerative motor neuron disease. BicD is autoinhibited from binding to Dynein in the absence of cargo. One such cargo is the RNA-binding protein Egalitarian (Egl). Egl in conjunction with BicD links mRNA transcripts including *osk*, *grk*, and *bcd*, to the Dynein motor. To identify how Egl or other cargos activate this complex, we used proximity biotin labeling to define the interactome of BicD in the presence and absence of Egl. In control strains, we found over 200 potential interacting partners of BicD. These interactions included several nucleoporins such as Nup358, a known cargo of mammalian BicD. In the absence of Egl, most of BicD's interactions, including Nup358, remained intact. However, BicD's interaction with Dynein was greatly reduced. To further elucidate the mechanism by which BicD activates Dynein, we generated two BicD mutants; L731A and R688A. BicD_L731A was previously shown to be defective for binding Egl and BicD_R688A corresponds to a site within mouse BicD1 that is required for binding mammalian Nup358. Our results indicate that whereas BicD_L731A is defective for Dynein activation and motility, BicD_R688A results in Dynein hyperactivity. Collectively, our results suggest that BicD cargos can differentially regulate the ability of this adaptor to bind and activate Dynein.

654T **SCAR and the Arp2/3 complex regulate polar myosin and plasma membrane organization in dividing neuroblasts.** Giulia Cazzagon, Chantal Roubinet, Buzz BaumMRC Laboratory of Molecular Biology

While the Formin-nucleated actomyosin cortex is known to control the changes in cell shape that accompany division, much remains to be discovered about the role of branched actin networks during this process. Drosophila neural stem cells, called neuroblasts, divide asymmetrically to produce a large and small daughter cell with different fates. This results from a basally-directed cortical flow that clears myosin from the apical cortex at the onset of anaphase, leading to apical cortical expansion, followed by basally-shifted cytokinesis. Here we use neuroblasts to elucidate the role of the actin nucleator Arp2/3 in this process. In doing so, we identify an apically localised pool of actin-rich protrusions that depend on the presence of the Arp2/3 complex, which is required for the precisely choreographed changes in cortical organisation. Furthermore, the nucleation promoting factor SCAR shows a polarized apical localization, and this factor also seems to be required for proper apical membrane patterning. Reductions in the activity of the Arp2/3 complex lead to defects in apical membrane dynamics and to increased accumulation of myosin in metaphase. At the onset of anaphase this results in a delay in the clearance of apical myosin, leading to cortical instability and membrane defects at cytokinesis. These data point to a role for the SCAR/Arp2/3 pathway in organization of the apical actomyosin cortex during asymmetric cell division.

655T **Long non-coding RNA *Hsr-omega* provides scaffolding for the nuclear domain B-body** SooBin An[1], Miranda Adams[2], Anton Bryantsev[11]Molecular and Cellular Biology, Kennesaw State University, [2]Emory University

The structural organization of the cell nucleus poses many intriguing questions. One of them is the organization of nuclear domains, sharp-bordered nuclear compartments concentrating a range of selected nuclear proteins. In this study, we used the recently discovered B-body as a model to investigate the mechanisms governing the formation of nuclear domains. B-body forms in the cellular precursors of flight muscles in developing *Drosophila* pupae. Prior to myoblast fusion, the splicing factor Bruno (Bru) concentrates in a single, large B-body but transitions into multiple smaller speckles and a diffuse nuclear pattern shortly after the commencement of myogenesis. We hypothesized that B-body must contain an RNA scaffold because Bru association with B-bodies is RNase-sensitive. Using Immuno-FISH, we identified RNA colocalizing with the B-body as the lncRNA *Hsr-omega*. Next, we tested the requirement of protein and RNA components for the integrity of the B-body. Genetic knockdown of the protein Bru did not perturb the size of B-body, as revealed by *Hsr-omega* FISH. In contrast, when *Hsr-omega* was removed via genetic deletion, the distribution of Bru was severely affected. Since *Hsr-omega* is expressed in a broader range of tissues than Bru, we conducted a misexpression study to test if it is possible to reconstitute the B-body outside flight muscles. Nuclei of the midgut epithelium look similar to flight muscle progenitors and express *Hsr-omega*; however, ectopically expressed Bru did not accumulate to form a B-body in them. In summary, our study demonstrates the importance of an RNA scaffolding for the B-body and highlights the importance of an additional mechanism to enable protein trafficking and accumulation at B-bodies.

656T ***Drosophila* Tak1, Tab2, and MyoVI function in protein autophagy** Erica Biven, Erika R GeisbrechtBiochemistry and Molecular Biophysics, Kansas State University

Maintaining the three-dimensional structure of proteins is crucial for proper function. Misfolded or denatured proteins must be restored to their original folded shape or degraded. For normal protein turnover, the correct cellular machinery must be recruited to degrade target proteins in the proteasome or by autophagy in the lysosome. When disrupted,

damaged proteins accumulate and cellular or organismal death can occur. In autophagic protein clearance in *Drosophila melanogaster*, the kinase NUAK and Starvin (Stv), the ortholog of the mammalian Bcl-2-associated athanogene 3 (BAG3), cooperate to recruit components of the autophagosome, including p62. The human homolog, NUAK1, belongs to a family of kinases known to be phosphorylated by four different upstream kinases: Liver kinase B1 (Lkb1), Ca2+/calmodulin-dependent PK kinase β (CaMKK), transforming growth factor-β-activated kinase 1 (Tak1), and serine/threonine kinase 38 (STK38). Muscle defects due to protein accumulation in Tak1 RNAi knockdown is exacerbated in a *NUAK+/-* heterozygous background. No significant defects resulted from knockdown of Lkb1, CaMKK, or the *Drosophila* homolog of STK38, called Tricornered (Trc). Tab2 is known to mediate the activation of Tak1. Tab2 knockdown produced muscle defects that were enhanced in a *NUAK+/-* background, indicating Tak1 and Tab2 function in autophagy. Knockdown of another protein implicated in autophagy, Myosin VI (MyoVI), causes mild muscle defects and increased p62 levels, both of which are exacerbated in a *stv+/-* background. These results together suggest Tak1, Tab2, and MyoVI function with the NUAK-Stv complex to regulate autophagy in muscle tissue.

657T **A Multiplayer game: how heteroplasmy transmission is regulated.** beitong gao, Ason C.Y. ChiangGenetics, Gurdon Institute, University of Cambridge

Mitochondrial diseases caused by mutations in the mitochondrial genome (mtDNA) affect 1 in 5000 individuals. There are multiple copies of mtDNA in an organism and different mtDNA variants can coexist, a phenomenon called heteroplasmy. The level of mutant mtDNA often determines the onset and severity of mtDNA linked disorders. Therefore, limiting the amount of detrimental mtDNA is vital for organismal health.

The nuclear genome is known to play an important role in governing mtDNA maintenance and transmission. However, very little is known about it influence the transmission of co-existing mitochondrial genomes. To identify nuclear factors that impact the competition among mtDNAs, we performed the first genome-wide haploid-insufficiency screen using *Drosophila* which stably transmits two mitochondrial genomes enforced by purifying selection benefiting one healthy genome and a selfish advantage favouring a detrimental mutant. This screen identified multiple nuclear loci that show a dosage effect on the heteroplasmy dynamics over generations. We mapped one locus to the catalytic subunit of mtDNA polymerase – *PolG1*. Interestingly, the reduction of PolG1 level (but not the rest of mtDNA replication machinery) significantly enhances the purifying strength that restrict pathogenic mtDNA mutations in offspring without altering the total mtDNA copy number (Chiang et al, Curr Biol 2019).

To extend the scope of our study, we established cultured *Drosophila* cells heteroplasmic for the same mtDNA variants. We are performing a genome-wide RNAi screen, which revealed some exciting modifiers of the heteroplasmy ratio already. We will carry out functional studies to reveal how they regulate heteroplasmy dynamics during somatic divisions. Our studies, using both *Drosophila* and cultured cell models, will gain a better understanding of heteroplasmy transmission during development.

658T **Regulation of pericentriolar material via Spd-2 C-terminal tail domain** Ryan S O'Neill[1], Frances C Welsh[2], Afeez Sodeinde[3], Carey J Fagerstrom[4], Brian J Galletta[4], Nasser M Rusan[41]National Heart, Lung, and Blood Institute, NIH, [2]UW, [3]Yale, [4]NHLBI

The centrosome is the major microtubule organizing center (MTOC) of the cell, ensuring proper spindle formation during cell division by recruiting pericentriolar material (PCM) proteins which in turn nucleate microtubules. In the most general sense, centrosomes are assumed to function similarly across cell types during division; however, a more nuanced view is emerging that PCM proteins and MTOC activity are differentially regulated across cell types, leading to functionally important diversity among different types of dividing cells. To gain insight into cell type-specific regulation of PCM proteins, we investigated a gene duplication of the PCM gene *Spd-2* using an evolutionary cell biological approach, reasoning that gene duplication could give rise to centrosome gene duplicates with cell type-specific functions. In *D. melanogaster*, *Spd-2* is a single copy gene, is ubiquitously expressed and functions by organizing PCM in both brain neuroblasts and spermatocytes. In contrast, *D. willistoni* has both *Spd-2A* (ancestral) and *Spd-2B* (derived). To explore expression and function we generated *D. melanogaster* animals with GFP-tagged *Spd-2A* and *Spd-2B* transgenes, including their *D. willistoni* native regulatory elements. Similar to Spd-2, Spd-2A organizes PCM in neuroblasts, but was insufficient for MTOC function in meiotic spermatocytes. In contrast, Spd-2B was only expressed in spermatogenesis, where it organized PCM during meiosis. Interestingly, driving expression of Spd2-B in the brain rescued neuroblast MTOC, but driving Spd-2A in spermatocytes still failed to rescue meiotic MTOC, indicating an evolutionary change in *Spd-2A* affecting meiotic function. We used chimeric transgenes to map these evolutionary changes to the ~120 amino acid

C-terminal tail domain of Spd-2A which was sufficient to prevent proper MTOC organization in meiosis. Together, these results indicate that somatic and germline cells have different requirements for PCM, and that Spd-2 is differentially regulated at the C-terminal tail to satisfy these requirements. Further, our evolutionary cell biological study points to a general model where the C-terminal tail of Spd-2 regulates protein levels in *D. melanogaster* to control PCM recruitment. Our current work aims to use additional chimeras, point mutants, and transgenes to gain further insight into this putative Spd-2 tail mechanism.

659T **Defining the role of prostaglandins within the substrate versus the migratory cells during collective cell migration** Samuel Mellentine, Anna Ramsey, Omar Rabab'h, Hunter Brown, Tina TootleAnatomy and Cell Biology, University of Iowa

Collective cell migration – the coordinated movement of associated cells – is important for both normal development and tumor invasion. One poorly understood regulator of cell migration in these contexts are prostaglandins (PGs), short-range lipid signals. To define the mechanisms whereby PGs regulate migration, we use collective migration of the *Drosophila* border cells to uncover the roles of PGs. During Stage 9 of oogenesis a cluster of epithelial cells becomes specified as border cells, delaminates from the epithelium, and migrates collectively and invasively between the nurse cells. Prior work found that loss of Pxt, the *Drosophila*cyclooxygenase-like enzyme responsible for all PG synthesis, results in delayed migration and decreased cluster cohesion. However, the particular PG or PGs controlling border cell migration remain unknown. To begin to address this, we assessed the roles of three PGE_2 synthases (mPGES-1, mPGES-2, and cPGES) and the sole $PGF_{2\alpha}$ synthase (PGFS/Akr1B) in border cell migration. Loss of cPGES or PGFS delays border cell migration but has no effect on cluster cohesion. These findings support the model that cPGES and PGFS are required for on-time border cell migration. We are currently using cell-specific RNAi knockdown to determine which cells produce PGE_2 and $PGF_{2\alpha}$. Initial studies suggest that cPGES acts in the nurse cells whereas PGFS acts in the border cells to promote border cell migration. We are also assessing downstream targets of each PGs. One known target ofPGs during border cell migration is integrin. We find that loss of PGFS phenocopies loss of Pxt, resulting in decreased localization of integrins to the border cell membrane. Together our data lead to the model that PGE_2 and $PGF_{2\alpha}$ are produced in different cells to promote on-time border cell migration, revealing PGs act not only in the migrating cell but also in the microenvironment to drive migration. As PG signaling is highly conserved, these studies provide critical insight into the specific functions of individual PG signaling cascades in controlling collective cell migration and can be applied to understanding both developmental collective cell migrations and pathological collective migrations.

660T **The nephrocyte actin and tubulin cytoskeleton networks model slit diaphragm structural defects pertaining to podocyte pathogenesis** Megan DelaneyEpidemiology and Human Genetics, University of Maryland-Baltimore

The human podocyte, found within the glomerulus of the kidney, functions as the filtration unit through which the protruding foot processes interlace with adjacent podocytes and create specialized cell junctions called slit diaphragms (SD). These slit diaphragms allow proteins and other substances to be filtered from the blood that are less than the size of serum albumin. Slit diaphragm defects, which can be caused by many nephrotic diseases, including chronic kidney disease (CKD) and Nephrotic Syndrome (NS), result in proteinuria. Previous research has indicated that the Actin Cytoskeleton Network (ACN) plays a key role in maintaining the foot process structure, but very little research has been previously conducted investigating the role of the Tubulin Cytoskeleton Network in the podocyte. The Drosophila nephrocyte shares a structure and function that is homologous to the podocyte. I used the nephrocyte as a model to investigate the cytoskeleton proteins in the maintenance of nephrocyte filtration function. I further studied the interaction between the ACN, TCN, their shared binding proteins, and the slit diaphragm. I show that tubulin genes are required for the organization of the TCN, ACN, and ultimately the SD, while actin genes are required for the SD formation but unnecessary for the TCN. Among the 14 cytoskeleton binding proteins, Pickled eggs (Pigs) and Karst (Kst) are important for SD structure. My study shows that the Drosophila nephrocyte is an excellent model to study the cytoskeleton network pertaining to the kidney filtration function. My research also sheds light towards understanding the pathogenesis of kidney diseases caused by cytoskeleton dysfunction.

661T **Pericentrin-Like-Protein is a Kinesin-1 Interactor That Drives Centriole Motility** Matthew R Hannaford[1], Rong Liu[2], Carey J Fagerstrom[1], Brian J Galletta[1], James R Sellers[1], Nasser M Rusan[1]1NHLBI, NIH, [2]School of Medicine, West Virginia University

Centrosomes are one of the primary organizers of the microtubule (MT) cytoskeleton within cells. They comprise a pair

of centrioles which act as a platform for a matrix of proteins termed the pericentriolar material (PCM). PCM is required for the nucleation and anchoring of MTs. Through MT organization, centrosomes are important for the mitotic spindle, cilia and flagella. To fulfill these varied functions, centrosomes must achieve proper positioning within the cell. Therefore, centrosomes must be motile. Typically, centrosomes are thought to position themselves via motor proteins pushing or pulling on the MTs anchored at the centrosome. However, in some cases, centrioles are motile without the presence of PCM or anchored microtubules, we refer to these as inactive centrioles. In this work we investigated how inactive centrioles are able to move through interphase cells. We reveal that these centrioles are cargo, being transported along the interphase microtubule array. We show that Kinesin-1 localizes to the centriole and is important for motility via an interaction between the Kinesin cargo binding tail and Pericentrin Like Protein (PLP); a coiled-coil rich protein which localizes to the outer centriole. Reverse yeast-2-hybrid screening allowed us to identify specific mutations which block the interaction between Kinesin-1 and PLP, live cell imaging then demonstrated that these mutations block interphase centriole motility. Reconstitution of this protein complex in vitro revealed that interaction with PLP is dependent upon the relief of Kinesin-1 autoinhibition. In this work we propose the first detailed mechanism of how centrioles can move independently of their role as an MTOC. We will further discuss our recent in vitro and in vivo efforts to dissect the mechanism of Kinesin-1 activation by kinesin activators, which promote PLP binding and efficient centriole motility.

662T **Different Actin Populations Determine How Cell Wounds Undergo Repair** Justin Hui[1], Julien Dubrulle[2], Mitsutoshi Nakamura[1], Susan M Parkhurst[11]Basic Sciences, Fred Hutchinson Cancer Center, [2]Fred Hutchinson Cancer Center

To carry out timely cell wound repair, actin and other cytoskeletal elements in the actin cortex must function in concert to form an actomyosin ring (AMR) to pull the wound closed. The formation of the AMR is coordinated in part by Rho family GTPases, which through their different downstream effectors regulate both linear and branched actin. We have previously shown the detrimental effects on wound repair when the cell is missing one or more Rho family GTPase members. Here, we investigate the contribution of each actin nucleator downstream of the Rho family GTPases on the organization of the AMR at the wound periphery. We show that formins (Dia and DAAM; linear actin nucleation factors) and Wiskott-Aldrich Syndrome proteins (WASp and SCAR; branched actin nucleation factors), along with their cofactor Arp2/3, play non-redundant roles in facilitating actin organization of the contractile AMR. We find that each of these actin nucleation factors exhibits different spatiotemporal recruitment patterns. In particular, the order of first recruitment to the injury is: Dia>DAAM/SCAR>WASp. Individual knockdown of each actin nucleator resulted in distinct wound repair phenotypes including differences in initial wound expansion, contraction rates, actin mesh densities, and actin filament orientations. Interestingly, we find that loss of DAAM, WASp, and SCAR, whose recruitment to wounds overlaps with that of the actin ring, resulted in significant changes to the orientation of actin filaments at the wound edge. In contrast, knockdown of Dia, which is recruited to the inner edge of the actin ring and inside of the wound, did not affect filament orientation; further demonstrating their non-redundant functions in cell wound repair. Although the actin cortex is severely disrupted in each of these knockdowns, wounds are still able to close, highlighting the resilience of repair mechanisms. Indeed, simultaneous removal of both linear and branched actin nucleators are needed to stop cell wound repair. Next, we disrupted myosin activity in the absence branched actin. Unexpectedly, these embryos exhibited primarily bundled linear actin filaments and employed a previously unknown mechanism of cell wound repair wherein linear actin filaments undergo a concerted chiral spiraling movement at the injury site to close the wound. Taken together, our results show that different actin filament populations are required to mediate optimal wound repair.

663F **A Dominant Modifier Screen for Genetic Interactors of Jagunal in the *Drosophila* Compound Eye** Laura Galvan[1], Gerson Ascencio[1], Judy Abuel[2], Jorge Alberto Inojoza[1], Grace Gundy[1], Alyssa Jimenez[1], Nhein Lu[1], Blake Riggs[31]Biology Department, San Francisco State University, [2]Biology Department, San Francisco State Univeristy, [3]Department of Biology, San Francisco State University

The Endoplasmic Reticulum (ER) is a continuous network of membrane tubules and flattened cisternae involved in protein and lipid synthesis, secretory proteins, and post-translational modification of proteins. A recent study identified a highly conserved protein, Jagunal (Jagn), which was found to play an important role in the asymmetric partitioning of the ER in pro-neural cells during mitosis in early embryonic development of *Drosophila* prior to cell fate determination. However, the pathway involving the role of Jagn in cell fate determination is poorly understood. We hypothesized that Jagn interacts with other genes that drive the generation of neural cell diversity and cell fate determinants. To identify the specific genes that interact with Jagn, we employed a genetic approach using a dominant modifier screen in the *Drosophila* compound eye. Here we expressed a JagnRNAi transgenic line inhibiting Jagn and displaying a rough eye phenotype. We then crossed a collection of deficiency lines covering the entire 3rd chromosome and examined progeny

for either an enhancement or suppression of the rough eye phenotype. Based on our screening efforts, we identified eight suppressors and ten enhancers of Jagn-induced rough eye phenotype. The deficiency lines identified as modifiers contained genes that we selected based on their involvement in functions such as organelle assembly, microtubule attachment, and organelle movements, which are important for ER function. Surprisingly, we identified a modifier gene, Presenilin (Psn), which is a transmembrane protein involved in proteolysis of the Notch pathway and is required for S3 cleavage that enables the release of activated Notch protein from the cell membrane. Based on these findings, we propose that Jagn interacts with Psn to modify the Notch signaling pathway driving cell fate selection. Future studies will examine the interaction between Jagn and other components of the Notch signaling pathway in order to better understand the role of the ER in the generation of cell diversity.

664F **Cytoophidia maintain the integrity of *Drosophila* follicle epithelium** Qiao-qi Wang[1], Dong-Dong You[2], Ji-Long Liu[21]School of Life Science and Technology, ShanghaiTech University, [2]ShanghaiTech University

CTP synthase (CTPS) forms a filamentous structure termed the cytoophidium in all three domains of life. The female reproductive system of *Drosophila* is an excellent model for studying the physiological function of cytoophidia. Here, we use $CTPS^{H355A}$, a point mutation that destroys the cytoophidium-forming ability of CTPS, to explore the in vivo function of cytoophidia. In $CTPS^{H355A}$ egg chambers, we observe the ingression and increased heterogeneity of follicle cells. In addition, we find that the cytoophidium-forming ability of CTPS, rather than the protein level, is the cause of the defects observed in $CTPS^{H355A}$ mutants. To sum up, our data indicate that cytoophidia play an important role in maintaining the integrity of follicle epithelium.

665F **“Mitotic” Kinesin-5 regulates axonal growth in the nervous system of *Drosophila*** Wen Lu, Margot Lakonishok, Helen Xue Ying Deng, Brad Lee, Jackie Crystal Wang, Vladimir I. GelfandCell and Developmental Biology, Northwestern University Feinberg School of Medicine

Kinesin-5 is a conserved homotetrameric motor that slides antiparallel microtubules apart in the bipolar spindle during mitosis. Here we find that kinesin-5 is essential for the proper development of postmitotic neurons in *Drosophila melanogaster*. The *Drosophila* kinesin-5 homolog, Klp61F, is expressed in the larval brain neurons, mostly abundantly in the ventral nerve cord (VNC) neurons. Knockdown of Klp61F by a pan-neuronal driver, *elva-Gal4*, results in severe locomotion defects and complete lethality in adult flies. These defects are mostly attributed to the lack of kinesin-5 in motor neurons in the VNC during the larval stages. Knockdown of Klp61F causes major axon growth defects both in culture and *in vivo*. The adult lethality and axon growth defects can be fully rescued with a human-*Drosophila* kinesin-5 chimeric motor that accumulates at the axon tip. Furthermore, we demonstrate that more microtubules penetrate into the actin-rich lamellipodia region in *klp61F-RNAi* knockdown cells. Altogether, we propose that kinesin-5 prevents microtubules from entering the periphery zone of the growth cone prematurely and thus is required for correct axon pathfinding during the nervous system development.

666F **Monitoring fatty acid trafficking in follicles reveals a critical role for DGAT1/Midway in protecting mitochondrial integrity** Roger P White, Michael A WelteBIOLOGY, University of Rochester

Circumstantial evidence suggests that lipids are crucially important for successful oogenesis, but the underlying mechanisms remain poorly characterized. During mid-oogenesis in Drosophila, lipoprotein particles in the hemolymph deliver fatty acids (FAs) to nurse cells. Here, the FAs are converted by the enzyme DGAT1 into triacylglycerides and stored in this form as lipid droplets (LDs). To directly monitor FA trafficking, we utilize fluorescently labeled fatty acids (FLFA). When isolated follicles are incubated in media containing fluorescently labeled palmitic acid (C16:0), lauric acid (C12:0) or arachidonic acid (C20:4) signal predominately appears in LDs and is detectable within 15 min, identifying LDs as a major destination for incoming FAs. Thin-layer chromatography of follicle extracts confirms that the FLFA is incorporated into neutral lipids, and we are currently employing MALDI-MS to identify the exact lipid species. When flies are fed food supplemented with FLFAs, FA trafficking is more complex. C12:0 accumulates in nurse cell LDs under all conditions tested, but when flies were starved prior to FLFA feeding, C16:0 does not enrich in LDs, but rather mitochondria. Thus, nurse cells appear to be able to regulate the ultimate destination of FAs depending on physiological state and type of FA. In *DGAT1* mutants, LDs are not produced and FLFA accumulates in mitochondria. We hypothesize that incoming FAs are routed by default to mitochondria when they cannot be sequestered in LDs. Intriguingly, *DGAT1* mutant follicles arrest in stage 8/9, and nurse cell mitochondria take on an abnormal morphology (toroidal), that is often associated with oxidative stress. Indeed, using MitoSOX, we find *DGAT1* mutants show higher levels of mitochondrial ROS than wild type (WT) follicles. We closely measured the oxygen consumption rate using Seahorse to find that *DGAT1* mutants have lower

oxygen consumption rate than in WT. We hypothesize that excess FA accumulation in mitochondria causes dysfunction. Alternatively, excess FAs may lead to the developmental arrest which secondarily results in mitochondrial defects. To distinguish between these possibilities, we are taking a genetic approach. We found that dosage reduction of the nuclear receptor Eip75B, a Drosophila PPARγ ortholog, allows *DGAT1* mutant follicles to develop as far as stage 10B. We are in the process of determining the mitochondrial phenotypes in the "rescued" Stage 9 follicles.

667F **Mechanisms of RNA localization to centrosomes** Junnan Fang[1], Rose Tian[1], Hala Zein-Sabatto[2], Dorothy A. Lerit[11]Cell Biology, Emory University School of Medicine, [2]Emory University School of Medicine

Centrosomes are microtubule-organizing centers tasked with maintaining mitotic fidelity, organizing the intracellular trafficking of cargoes, and building cilia. Contributing to the diversity of centrosome functions are cell cycle-dependent oscillations in microtubule-nucleating activity instructed by the pericentriolar material organized around a central pair of centrioles. Also localizing to centrosomes are mRNAs. While RNA was long known to localize to centrosomes, conflicting early reports cast its physiological significance into doubt. In the intervening decades, centrosomal RNAs remained understudied. Recent work from our group and others suggests the functional role of mRNA residing at centrosomes is of emerging importance. Increasing evidence implicates centrosomes as sites for local protein synthesis and translational regulation. The early *Drosophila* embryo is a powerful model for investigating RNA localization to centrosomes due to its transcriptional quiescence and relative tractability for manipulating RNA localization. For example, we recently demonstrated the translational repressor FMRP regulates *centrocortin* (*cen*) mRNA localization and protein abundance required for error-free mitosis in early *Drosophila* embryos. Mislocalization of *cen* mRNA to the anterior cortex abrogated Cen protein localization to distal centrosomes and led to mitotic defects, demonstrating a requirement for *cen* mRNA localization to centrosomes. Moreover, centrosome localization of *cen* and other mRNAs, such as *pericentrin-like protein* (*plp*) mRNA, is puromycin-sensitive. While these data suggest RNA localization to centrosomes occurs via a co-translational transport mechanism, precisely how mRNAs are trafficked to centrosomes remains unknown. Our work demonstrates RNA enrichments at the centrosome are regulated developmentally and cell cycle stage-dependent, strongly hinting that RNA localization to the centrosome is an active process. We will discuss our unpublished data investigating roles of microtubules, the dynein motor complex, and identifying RNA-binding proteins and *cis*-sequences required for mRNA localization to centrosomes. By identifying the *cis*- and *trans*-factors required for mRNA localization to centrosomes, we aim to elucidate paradigms of centrosome regulation, features of which may be deregulated in in human pathologies associated with centrosome dysfunction, such as microcephaly, ciliopathy, and cancer.

668F **The G-Signaling Protein Rcp Controls the Polarized Basement Membrane Deposition in Epithelial Cells** Lindsey Price[1], Trent Davids[1], Rebecca Brnot[1], Alejandro Salas[1], Tracie Yiging Kong[2], Trudi Schupbach[2], Olivier Devergne[11]Biological Sciences, Northern Illinois University, [2]Molecular Biology, Princeton University

Epithelial tissues are the most common type of tissue in the human body, forming the outer layer of the skin and most organs. They are composed of epithelial cells and rely heavily on their cellular architecture. This architecture is organized by an apical-basal polarity, one critical component of which is the proper placement of the basement membrane (BM). The BM is a specialized sheet within the extracellular matrix lining the basal side of epithelial cells. It is a vital structure for the establishment and maintenance of the epithelial cell architecture. Importantly, the loss of integrity and mis regulation of the BM have been associated with pathological situations, including cancer. The biological pathway dedicated to the proper placement of the BM controls the production of BM proteins inside epithelial cells and their specific secretion to the basal side of these cells. Despite the BM's important role in epithelial cell organization and polarity, the biological pathway dedicated to the polarized secretion of BM proteins is poorly understood. To study BM deposition, we use the follicular epithelium (FE) of the *Drosophila* ovary as a model system. In a genetic screen looking for 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. The loss of *Rcp* leads to aberrant apical deposition of BM proteins, such as Perlecan, Collagen IV and Laminin, in FE cells. However, the loss of *Rcp* does not primarily affect the localization of apical and lateral polarity proteins. This indicates that *Rcp* specifically controls the proper placement of BM proteins. Interestingly, previous genes found to be important for the proper placement of BM proteins are involved in trafficking, however *Rcp* is the first component of a signaling pathway that has been implicated in BM polarity. Finally, using immunostaining and super-resolution microscopy, we determined that *Rcp* localizes in the cytoplasm and nucleus of epithelial cells. Altogether, our results uncover a specific role for *Rcp* in the organization of epithelial architecture by regulating the polarized deposition of BM proteins, a critical process in the establishment and maintenance of epithelial architecture.

669F **Design and characterization of optogenetic tools to manipulate Rap1 GTPase activity during collective cell migration** Yujun Chen[1], Katheryn Rothenberg[2], Rodrigo Fernandez-Gonzalez[2], Jocelyn McDonald[11]Division of Biology, Kansas State University, [2]Institute of Biomedical Engineering, University of Toronto

Collective cell migration is essential in many developmental and pathological processes. Despite extensive research conducted in a variety of models, the mechanisms underlying collective cell migration especially within intact tissues and organs are still poorly understood. *Drosophila* border cells travel as a cohesive cluster during oogenesis and provide an excellent genetic model for identifying how cell collectives move inside tissues. While the roles of the small GTPase Rap1 in cell migration have been investigated in several different tissues, it is unclear how Rap1 spatially and temporally influences collective cell migration. Here, by taking advantage of the improved light-inducible dimer (iLID) and its binding partner SspB, we developed Opto-Rap1-On and Opto-Rap1-Off systems for spatiotemporal control of Rap1 activity. The Opto-Rap1-On consists of two components: a light-activatable membrane anchor (Venus-iLID-CAAX) and a Rap1 activator (tRFP-SSPB-C3Gcat); for the Opto-Rap1-Off, Venus-iLID-CAAX and an inactivator for Rap1 (tRFP-SSPB-RapGap1cat) were used. Overexpression of only Venus-iLID-CAAX, tRFP-SSPB-C3Gcat, or tRFP-SSPB-RapGap1cat using the GAL4-UAS system had no off-target effects on collective border cell migration, indicating the system is not leaky. We also were able to successfully recruit the tRFP-SSPB-RapGap1cat to the cell membrane from the cytoplasm after induction with blue light in S2 cells and border cells *in vivo*. To determine the spatial-temporal activity of Rap1, we further developed a Rap1 activity biosensor. This biosensor faithfully detected the increased Rap1 activity caused by overexpression of a constitutively active Rap1 mutant. We are currently optimizing the expression level of tRFP-SSPB-C3Gcat in border cells to test its recruitment to the cell membrane and to activate Rap1. We are also testing if our designed Rap1 biosensor effectively monitors endogenous Rap1 activity *in vivo*. We will report our progress in evaluating the functionality of the Opto-Rap1-On and Opto-Rap1-Off systems by taking advantage of the Rap1 biosensor and our further improvements to these tools. Our expectation is that these tools will be valuable in dissecting the acute functions of Rap1 in many tissues, as well as in collective cell migration specifically.

670F **Glial-specific knockdown of a subunit of the ER membrane complex (EMC) dramatically reduces survival of *D. melanogaster*** Maria Jose Orozco Fuentes[1], Otoha Tatami[1], Rebecca Delventhal[21]Biology, Lake Forest College, [2]Lake Forest College

The endoplasmic reticulum (ER) is involved in the modification, packaging, and insertion of membrane proteins. The ER membrane protein complex (EMC) is believed to facilitate many ER functions related to protein biogenesis. This protein complex is highly conserved from yeast to humans, and it is composed of 8-10 subunits that work in tandem. Through an RNAi knockdown screen, we discovered that glial-specific knockdown of one subunit, EMC4, resulted in severe phenotypes. We observed delayed development, strongly impaired locomotion, a lifespan of only 5-6 days, and increased protein aggregation. The dramatic effect of glial-specific loss of EMC4 highlights the importance of this subunit to development and organismal health, while raising intriguing questions about its cell-specific function. Future studies on the effect of tissue-specific knockdowns other than glia will offer additional insight about potential cell type specific-protein-processing mechanisms in the ER. Additionally, temporal restriction of EMC4 knockdown to specific developmental stages will provide an understanding of when the EMC is required during development.

671F **Using *Drosophila* denticles as a model system to investigate the role of cytoskeletal proteins in the formation of actin-based protrusions** Jennifer L SalleeBiology, North Central College

The formation of actin-based protrusions, such as the denticles in *Drosophila melanogaster*, requires the coordination of extensive actin-associated proteins to crosslink and bundle actin filaments. Mutations in such proteins can cause defects in the shape, structure, and function of actin-based protrusions and provide us with information on the molecular mechanisms of their function. Often, mutations in genes of the cytoskeleton can be lethal in adults but mutant flies can survive through embryogenesis allowing for denticles to be an excellent model system for studying the function of these genes. Our goal was to establish a method for systematically examining denticle size and shape to compare the function of a variety of cytoskeletal genes in the formation of actin-based protrusions. Here, we examined the mutants of several genes known to cause bristle and wing hair defects for their effect on denticle morphology. Cuticle preps of late-

stage embryos from loss of function alleles of *singed, forked*, *twinfilin*, *flare*, *twinstar*, and *ck*/MyoVIIA were examined for their overall denticle morphology (shape) as well as for the height and widths of the denticles. Single mutants, *ck;twinfilin* and *ck;flare* double mutants, and *sn,f;ck* triple mutants were compared to determine if combinations of the genes might show genetic interactions. Although severe phenotypes were observed in bristles and wing hairs, *singed, forked*, *twinfilin, twinstar,* and *flare* mutants showed mild denticle defects suggesting that the stage of development and potential interactors present at that stage might influence the effect on the actin-based protrusions.

672F **The STRIPAK complex and autophagy in *Drosophila* muscle tissue** Yungui Guo, David Brooks, Erika GeisbrechtBiochemistry and Molecular Biophysics, Kansas State University

In humans, Bcl-2-associated athanogene 3 (BAG3) is essential for proteostasis in stressed cells. Some of the known functions of BAG3 include promoting chaperone activity, facilitating aggresome formation, and initiating the destruction of proteins via autophagy. Our lab has discovered that *Drosophila* Starvin (Stv), the ortholog of mammalian BAG3, biochemically and genetically interacts with the evolutionarily conserved NUAK serine/threonine kinase, and this NUAK-Stv complex plays a role in the autophagic clearance of proteins in larval contractile muscles. Using an in silico approach, we identified Striatin interacting protein (Strip), MOB kinase activator 4 (Mob4), and Fibroblast growth factor receptor 1 oncogene partner 2 (Fgop2) as conserved mediators of muscle tissue maintenance. We performed affinity purification mass spectrometry (AP-MS) experiments with Strip as a bait protein and co-purified additional Striatin Interacting Phosphatase and Kinase (STRIPAK) complex members from larval muscle tissue. Interestingly, NUAK and Stv also emerged as Strip-binding proteins and these physical interactions were verified in vivo using Proximity Ligation Assays (PLA). To understand the functional significance of the STRIPAK-NUAK-Stv complex, we employed a sensitized genetic assay combined with RNA interference (RNAi) to demonstrate that both *NUAK* and *stv* act in a pathway with genes that encode for STRIPAK complex proteins. *Strip RNAi* muscles showed elevated Atg8a puncta and increased p62 protein levels, suggestive of decreased autophagic flux. Indeed, reduced Strip protein levels impeded autophagy at the autophagosomal-lysosomal fusion step. Surprisingly, we also observed the abnormal accumulation of endocytic vesicles marked by Rab7 in *Strip*, *NUAK*, or *stv RNAi* muscles. Since defects in endocytic maturation can compromise autophagic function, our results together support a model whereby the STRIPAK-NUAK-Stv complex coordinately regulates aspects of both endocytosis and autophagy for efficient autophagic flux via lysosomal degradation.

673F **Establishing a procedure for the enrichment of circulating exosomes from *Drosophila* hemolymph** Akimi Green, Young KwonBiochemistry, University of Washington

The study of extracellular vesicles (EVs) has the potential to discover molecular mechanisms involved in intercellular and interorgan communication during homeostasis and disease. Exosomes are a subset of EVs, ranging in size from 40-160 nm, that primarily exist in bodily fluids, such as blood. To study circulating exosomes *in vivo*, it is important to establish a procedure for the reliable isolation of exosomes from an animal. We have successfully established a procedure for enriching exosomes from larval hemolymph by adapting procedures for isolating exosomes from mammalian cultured cells. Briefly, our protocol involves the collection of hemolymph from 3rd instar larvae and centrifugation at low speeds to pellet cells, debris, and large vesicles. Then, we subject the supernatant to a series of high-speed 100,000xg spins to pellet the exosome-sized vesicles, separating exosomes and lower-density particles, such as lipoproteins. This fraction, named P100, was subsequently analyzed via particle analysis and SDS-PAGE. We found an enrichment of particles between 60-150 nm. Further, when run on SDS-PAGE, we observe a unique protein “fingerprint” associated with the exosome-enriched P100 compared to the whole hemolymph. These results suggest that we have established a reliable protocol for isolating exosome-sized vesicles from larval hemolymph. A recently published study has independently established a procedure for enriching exosomes in larval hemolymph. We will compare two procedures mainly using particle analysis. Next, we will utilize CD63-GFP, a known exosome marker, to further test whether CD63 is present in this P100 fraction.

674F **Fat2 organizes an interface signaling system that directs collective epithelial cell migration** Audrey Williams, Sally Horne-BadovinacUniversity of Chicago

Collective cell migration is a major part of the morphogenetic toolkit of animal epithelial cells. Epithelial cells remain adhered to neighboring cells as they migrate, necessitating a level of cell-cell coordination for efficient collective motion. One recurring coordination motif is the enforced polarization of individual cell-cell interfaces such that a leading edge is always positioned opposite another cell’s trailing edge. This helps align the motility apparatus of neighboring cells. In this study, we ask how leading-trailing interface polarization is achieved in the follicular epithelium of the *Drosophila*

egg chamber, in which the follicle cells undergo a collective migration that strongly depends on interactions between neighboring cells. In this tissue, a suite of transmembrane signaling proteins is polarized to leading-trailing interfaces and required for normal migration. These proteins include the atypical cadherin Fat2, which is enriched at trailing edges, and the receptor tyrosine phosphatase Lar and the semaphorin Sema-5c, which are enriched at leading edges. Loss of either Lar or Sema-5c slows migration, and loss of Fat2 prevents it entirely. Using genetic and pharmacological manipulations, live imaging, and quantitative image analysis, we investigated how these proteins become polarized, and how they contribute to polarization of the cell motility apparatus and to broader collective migration. Fat2, Lar, and Sema-5c all colocalize in interface-spanning puncta along with the protrusion-promoting WAVE complex. We found that Fat2 is required in trans to recruit Sema-5c to these puncta, as it was previously shown to recruit Lar. Probing the dynamics of the puncta with FRAP or acute Fat2 inhibition, we observed that Fat2 molecules are relatively stable, and are continuously required to maintain enrichment of more dynamic Lar and Sema-5c molecules on the other side of the interface. Once recruited by Fat2, Lar and Sema-5c act in parallel to regulate distinct aspects of follicle cell migration. Synthesizing phenotypic analysis from this study with published findings, we propose that Lar acts in cis to promote protrusion alignment in the direction of tissue movement, whereas Sema-5c signals in trans to the trailing edge to promote trailing edge-like or restrict leading edge-like behavior there. Together, these activities ensure the juxtaposition of leading and trailing edges, and therefore promote coherent collective migration.

675F ***Hobbit* is a novel and conserved regulator of tissue growth and apoptosis** Sarah D Neuman, Arash BashirullahPharmaceutical Sciences Division, University of Wisconsin-Madison

The bridge-like lipid transfer proteins (BLTPs) are a novel and conserved family of proteins that form long hydrophobic channels bridging organelle membranes at membrane contact sites, where they function as non-vesicular lipid transporters. We initially identified *hobbit*, one member of the BLTP family, in a forward genetic screen for metamorphosis-specific lethal mutations with a small body size in *Drosophila*. Loss of *hobbit* function impairs regulated exocytosis of insulin, leading to the small pupa phenotype. The Hobbit protein itself localizes to endoplasmic reticulum-plasma membrane (ER-PM) contact sites and binds to phosphoinositide lipids (PIPs); additionally, the subcellular distribution of at least one PIP, $PI(4,5)P_2$, is disrupted in *hobbit* mutant cells. Interestingly, the human ortholog of *hobbit* (*BLTP2*) is overexpressed in a variety of human cancers, leading us to test if overexpression of *hobbit* results in any cancer-like phenotypes in *Drosophila*. We found that overexpression of *hobbit* in the wing imaginal disc sometimes causes dramatic overgrowth. Furthermore, *hobbit* overexpression strongly inhibits caspase activation in response to an apoptotic stimulus in this same tissue. In contrast, one copy loss of *hobbit* dramatically increases sensitivity to apoptotic triggers. Together, these results suggest that *hobbit/BLTP2* may be a novel driver of oncogenesis. Our current goal is to determine how a non-vesicular lipid transporter contributes to tissue overgrowth and resistance to apoptosis.

676F **The *Drosophila* EGFR ligand mSpitz is delivered to cytoplasmic capes at sites of non-canonical RNA export on the nuclear envelope *via* the endosomal system** Floyd. J Mattie[1], Praveen Kumar[2], Mark. D Travor[1], Kristen C Browder[3], KINSUK SHILL[1,1], Claire Thomas[11]The Pennsylvania State University, [2]Government College for Women, [3]Genentech Inc

Nuclear-cytoplasmic communication is not limited to nuclear pores, with both proteins and RNA using alternative routes between these compartments. We previously characterized cytoplasmic capes (large invaginations of the nuclear envelope in *Drosophila*), that are enriched for the membrane-bound EGF receptor ligand mSpitz, endosome-related organelles and ubiquitylated proteins. Closely associated with capes are groups of perinuclear vesicles between the outer and inner nuclear membranes that resemble those seen at sites of non-canonical ribonucleoprotein (RNP) export *via* budding. Here, we demonstrate that mSpitz delivery to nuclear capes requires passage through the endosomal system. We also show that capes are indeed closely associated with sites of non-canonical RNP export as well as the *D*Frizzled2 receptor C terminal fragment (*D*Fz-2c), a core component of this export pathway. Time-lapse microscopy of glands in intact larvae indicates that cytoplasmic capes are stable structures that persist for at least 90 minutes without conspicuous growth. We further show that capes appear with the growth of the salivary gland rather than at a specific developmental stage, suggesting that capes do not form in response to ecdysone signaling. We also demonstrate that the large F-actin binding protein β_H-spectrin that modulates endosomal trafficking, as well as spectrin tetramer formation are required for cape formation. Finally, we find that there is a slight elevation in the level of *D*Fz-2c at the nuclear envelope when β_H is knocked down. Cytoplasmic capes therefore represent a sub-specialization of the nuclear envelope where endosomal trafficking and RNP export are closely associated and may be functionally interdependent.

677F **Nucleolar Stress in *Drosophila* Neuroblasts: Modeling Human Ribosomopathies** PATRICK J DiMarioLouisiana

State University

Nucleolar stress is a failure to produce functional ribosomes in sufficient quantities for protein synthesis and thus cell viability. Different cell types have variable thresholds for adequate ribosome production. For example, human embryonic neural crest cells or adult bone marrow stem cells are particularly sensitive to the loss of functional ribosomes below their requisite thresholds. This leads respectively to the Treacher Collins syndrome (craniofacial birth defects) or to Diamond-Blackfan anemia in adults. We induce nucleolar stress in *Drosophila* by depleting the ribosome assembly factor, Nopp140. Larvae homozygous for the CRISPR-disrupted *Nopp140* gene (in 78F4 of 3L) develop to the second instar stage where they can linger for several days before expiring. RT-PCR showed continued synthesis of the External Transcribed Spacer (ETS) and the Internal Transcribed Spacer 2 (ITS2) within the pre-rRNA in these *Nopp140-/-* larvae. While nucleoli in *Nopp140-/-* larvae remained intact, there was a substantial loss of cytoplasmic ribosomes in most cell types examined by TEM. Most neuroblasts (Type 1 and 2) in 2-day *Nopp140-/-* larvae failed to label with anti-Deadpan or EdU, indicating these neuroblasts exited the cell cycle upon nucleolar stress. However, mushroom body neuroblasts (MB NBs) and their lineage ganglionic mother cells (GMCs) in day-2 *Nopp140-/-* larvae maintained anti-Deadpan and EdU labeling suggesting they remained relatively resilient to the loss of Nopp140. From previous work, we know that Fibrillarin, the pre-rRNA methyl-transferase in C/D-box snoRNP, redistributes from nucleoli to the nucleoplasm upon loss of Nopp140, and we saw similar redistributions of Fibrillarin in the *Nopp140-/-* Type 1 and 2 neuroblasts. Fibrillarin remained within nucleoli of the MB NBs in *Nopp140-/-* larvae, suggesting their nucleolar function remained intact. We are currently testing for the involvement of JNK signaling in *Drosophila* nucleolar stress. To understand why MB NBs and GMCs are resilient to nucleolar stress in 2-day *Nopp140-/-* larvae, we are testing for maternal ribosome assembly factors preferentially stock-piled in MB NBs. Also, ribosomes have a life span of 3 days. So we are differentially tagging maternal and zygotic ribosomes to test if maternal ribosomes are stock-piled preferentially in certain embryonic and larval cells relative to increasing numbers of zygotic ribosomes.

678F **A Kinesin-like Protein Encoded by *CG14535* Controls Border Cell Migration During *Drosophila* Oogenesis**
Leif Verace[1], Lauren Anllo[2], Vivian Qin[3], Trudi Schüpbach[3], Olivier Devergne[11]Department of Biological Sciences, Northern Illinois University, [2]Department of Biology, East Carolina University, [3]Department of Molecular Biology, Princeton University

Cell migration plays a key role in many biological processes including embryonic development, tissue morphogenesis, and wound healing. Cell migration is also involved in metastasis, a hallmark property of cancer cells. Thus, understanding how this behavior works is of high importance. A well-established model system for studying migratory cell behavior is the migration of border cells (BCs) during *Drosophila* oogenesis. Oogenesis is an intricate process within *Drosophila*— a collection of nurse cells nurtures a growing oocyte, all surrounded by a monolayer follicular epithelium which forms the egg chamber (the future egg). BCs, originating from the follicular epithelium, are recruited as a cluster of epithelial cells which delaminate and migrate from the anterior to posterior end through the center of the egg chamber. This cellular behavior has been used as a model to study key pathways involved in cell motility. In a genetic screen looking for novel genes involved in BC migration, we identified a mutant allele *KC53* which results in border cell migration defects. This mutation was mapped to a kinesin-like gene, *CG14535*, on chromosome 2L. I will describe the different approaches and preliminary results we have obtained to investigate the role of *CG14535* in the control of BC migration. These include RNAi knockdowns, mutant clonal analysis, and the generation of a *CG14535* knockout using CRISPR/Cas9 genome editing to produce an amorphic mutant allele—which will allow us to further decipher the role of *CG14535* in the control of BC migration.

679F **Deciphering the link between CNK and Misshapen during thorax closure in *Drosophila*** Eloïse Duramé, Caroline Baril, Malha Sahmi, Marc TherrienUniversité de Montréal

Connector enhancer of KSR (CNK) is a scaffold protein conserved across metazoans that is critical for RAS-ERK signalling in Drosophila. However, the role of its mammalian counterparts is less clear. To further define the function of CNKs, we used the BioID technique to delineate their proximal interactomes in mammalian cells. Interestingly, several interactors identified are involved in cell motility, polarity and adhesion. In addition, all three members of the Misshapen family of kinases were identified. These kinases are known regulators of cytoskeletal events involved in epithelial morphogenesis. We sought to use Drosophila to decipher the link between CNK and the unique fly homolog of Misshapen, called MSN. Using S2 cells and co-immunoprecipitations assays, we first demonstrated that the CNK-MSN interaction is conserved in flies. We then investigated whether CNK and MSN could function in the same developmental event. Previous studies had shown the involvement of MSN in dorsal closure of the embryo and thorax closure during pupariation. Interestingly,

using the *pannier-Gal4* (*pnr-Gal4*) driver, *cnk* knockdown during thorax development led to thorax closure defects, reminiscent of the phenotype observed with *msn* depletion. Significantly, the cleft phenotype induced by *cnk* or *msn* RNAi was enhanced by crossing in, respectively, heterozygoys *msn* or *cnk* mutant alleles, demonstrating a genetic interaction. During pupal development of the thorax, the two epithelial halves of the wing discs migrate toward each other and fuse at the presumptive midline. Disruption of cytoskeletal events, largely link to JNK signalling, are known to alter this process, leading to a visible cleft in the dorsal midline. Time-lapse imaging of thorax closure showed that *cnk* or *msn* depletion prevented the fusion of the migrating discs. Moreover, *cnk* knockdown selectively reduced cell velocity. In parallel, we used miniTurboID to identify putative factors in S2 cells working together with CNK and MSN. This approach delineated 72 interactors common to CNK and MSN, many of which are involved in vesicular trafficking, cytoskeletal organization, polarity and migration. We are currently testing for genetic interactions between several of these candidates and *cnk* or *msn* in thorax closure. Together, this work suggests a role for CNK in epithelial events related to MSN activity in Drosophila. Its characterization should refine our understanding of the molecular events underlying epithelial morphogenesis.

680S **Differential roles for GSK3β and ERK kinases in a *Drosophila* model of Huntington's disease** Shermali Gunawardena[1], Thomas Krzystek[2], Rasika Rathnayake[2], Jia Zeng[21]SUNY at Buffalo, [2]Biological Sciences, SUNY at Buffalo

Huntington's disease (HD) is a devastating neurodegenerative disorder that manifests from an N-terminal polyQ-expansion (>35) in the Huntingtin (*HTT*) gene leading to axonal degeneration and significant neuronal loss. Despite evidence for a scaffolding role for HTT at membrane-related processes such as endocytosis, vesicle transport, and vesicle fusion, it remains unclear how mutant HTT alters membrane associations. By combining high-throughput proteomics in normal and HD patient iPSC derived neurons with a *Drosophila* model of HD and pharmacological and genetic inhibition, we found intriguing changes to the kinome of pathogenic HTT-containing membranes in HD patient neurons. In *Drosophila* GSK3β and ERK kinases showed opposing effects on mutant HTT-mediated phenotypes. Inhibition of GSK3β decreased pathogenic HTT-mediated axonal transport defects, synaptic dysfunction and neuronal cell death, while inhibition of ERK enhanced these defects. Intriguingly while GSK3β can phosphorylate HTT, enhanced GSK3β-mediated phosphorylation was observed in pathogenic HTT. Taken together, we propose that the ERK mediated effects are likely specific to the axonal transport of HTT, while GSK3β may influence the function of molecular motors. Since both GSK3β and ERK are predicted to phosphorylate HTT at the same Ser2657 site, our work uncovers a novel mechanism whereby GSK3β and ERK phosphorylation events likely play differential roles during pathogenic HTT-mediated neuronal dysfunction in HD.

681S **Quantitative proteomic analysis uncovers the specific vs general mitochondrial effects caused by mutants affecting three different critical pathways for mitochondrial function in Drosophila** Aditya Sen, Rachel CoxUniformed Services University

The majority of cellular ATP is produced in mitochondria, and functional mitochondria are vital for maintaining proper cellular homeostasis. Dysfunctional mitochondria affect almost all major organs in the body. Mitochondria contain their own DNA, mitochondrial DNA, which encodes thirteen proteins. However, mitochondria require thousands of proteins encoded in the nucleus to carry out their many functions. Mutations in one of these genes can cause a wide spectrum of abnormalities in animals. Despite the challenges in studying the mitochondrial proteome due to the dynamic nature of mitochondria, it holds the potential to identify both the specific and general mitochondrial damages caused by single gene mutations. To identify specific versus general effects, we have taken advantage of mutations in three Drosophila genes, *clueless* (*clu*), *Superoxide Dismutase 2* (*Sod2*), and *PTEN induced kinase 1* (*Pink1*), which are required for mitochondrial function through different molecular mechanisms. Clu is a nucleus-encoded ribonucleoprotein that forms mitochondrion-associated particles. *clu* mutants live less than a week and CluH knockout mice live only one day after birth, with both mutants exhibiting mitochondrial damage. Sod2 scavenges mitochondrial free radicals and mutants have increased oxidative damages. Pink1 is a component of the mitophagy pathway used to cull damaged mitochondria. *pink1* fly mutants are reduced lifespans and mitochondrial damage and mutations in PINK1 in humans is linked to early onset parkinsonism. Takin one-day-old flies, we measured changes in each mutant's mitochondrial proteome using quantitative tandem mass tag mass spectrometry. Our analysis identified protein classes that are unique to each mutant and those that are shared between them, suggesting that some changes in the mitochondrial proteome are due to general mitochondrial damage whereas others are gene specific. All three mutants induce a general mitochondrial stress response, however, *clu* mutants predominantly affect mitochondrial respiratory chain proteins as well as proteins involved in vesicle transport and cytoskeletal organization. For the first time, we have directly compared the in vivo steady-state levels of mitochondrial proteins in mutants affecting three pathways critical for mitochondrial function.

These data could be useful to understand disease etiology, and how mutations in genes critical for mitochondrial function cause specific mitochondrial proteomic changes as opposed to changes due to generalized mitochondrial damage.

682S **Investigating the function of Kibra and Merlin during border cell migration** KathyAnn Lee[1], Richard Fehon[21]Committee on Development Regeneration and Stem Cell Biology, University of Chicago, [2]Department of Molecular Genetics and Cell Biology, Committee on Development Regeneration and Stem Cell Biology, University of Chicago

Border cell migration is a fascinating part of Drosophila oogenesis. During mid-oogenesis the border cells and anterior polar cells delaminate from the follicular epithelium and collectively migrate between the nurse cells to reach the oocyte. In order for this to occur, the group of cells must maintain adhesion to one another and coordinate cytoskeletal rearrangements so that the leading cells extend out protrusions and the trailing cells retract their membranes. Previous work has found that several components of the Hippo signaling pathway, which has been best studied in the context of tissue growth, are required for this migration. However, Yorkie, the transcriptional effector of the Hippo signaling, is not required, suggesting that the effects of Hippo pathway activity on border cell migration are non-transcriptional. We are interested in potential functions of Kibra and Merlin, upstream components of the Hippo pathway, during this process. Previous analysis using fixed tissues showed that Kibra and Merlin are localized to cell-cell contacts within the border cell cluster, and absent from the leading and trailing edges migrating cell cluster. Using live imaging of endogenously expressed tagged proteins we have found that Kibra is polarized to the leading edge of the cells, a localization that was not apparent from previous studies in fixed tissue. We plan to determine if Kibra and Merlin contribute to border cell polarity and migration by exploring interaction with polarity components and the cytoskeleton. More generally, these studies should provide a broader understanding of how the highly conserved Hippo pathway coordinates tissue growth and morphogenesis.

683S **An Immunoglobulin cell adhesion junction module maintains epithelial integrity.** Tara M Finegan[1], Christian Cammarota[2], Dan T Bergstralh[11]Biology, University of Rochester, [2]Physics, University of Rochester

Epithelial cells can be born protruding from an epithelial tissue layer - without a connection to the basement membrane - then reincorporate into it. This process, called cell reintegration, appears to be a general property of epithelia. In flies, reintegration relies on a suite of adhesion proteins - Neuroglian, Fasciclin II, and Fasciclin II – that are distinct from the classical adhesion and occluding junctions. These proteins are not only found in proliferating epithelia but also in neurons, where they help drive axon fasciculation. Our model is that both fasciculation and reintegration are haptotactic, meaning that cell shape/movement is driven by adhesion. This raises the obvious question of why multiple factors are necessary. Does reintegration A) utilize a multivalent reintegration machinery that includes Nrg, Fas2, and Fas3, or does it B) depend on an amount of adhesion to which each of these proteins contribute? We are answering this question with a combination of structure-function analysis, genetic rescue experiments, and advanced imaging techniques.

684S **A screen of Serendipity-a interactors during cellularization identifies Dah and Slik** Matthew Kim[1], Leeza Turchin[2], Natalie Biel[3,4], Anna Sokac[31]Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, [2]Integrative Biology, University of Illinois at Urbana-Champaign, [3]Cell and Developmental Biology, University of Illinois at Urbana-Champaign, [4]Integrative Molecular and Biomedical Sciences, Baylor College of Medicine

Cellularization is a critical tissue-building event in the early *D. melanogaster* embryo and is dependent on the actin cytoskeleton. Serendipity-a (Sry-a) is an actin binding protein that is required for cellularization and positively regulates actin filament levels during cellularization. However, we do not understand how Serendipity-a provides this function at a molecular level, or what its interaction partners are. Publicly available yeast two-hybrid data suggests a number of candidate interactors that might provide more insight into Serendipity-a function. The goal of this study was to identify which candidate interactor proteins, when knocked down, mimic the *sry-a* mutant phenotype of furrow regression during cellularization. To examine knockdown phenotypes in cellularizing embryos for nine candidates, we used maternally inducible RNAi, with available TRiP line stocks crossed to germline drivers. Knockdown of transcripts was confirmed by RT-PCR and phenotypes were scored by immunofluorescence and confocal imaging. Negative and positive controls were included to validate the success of all procedures, such as Oregon-R (Ore-R) to show wild-type cellularization phenotype and mRNA levels; and *sry-a*$^{-/-}$ mutants and *slingshot* (*ssh*) RNAi knockdown to show furrow regression phenotype and RNAi-mediated reduction in mRNA level, respectively. We found two candidate interaction partners, *discontinuous actin hexagon* (*dah*) and *sterile20-like kinase* (*slik*), that when knocked down gave the same

furrow regression phenotype as *sry-a* mutants. Dah was previously shown to play a role in cellularization, but Slik is a new player. Slik is the *Drosophila* homolog of human STK10 (Sopko *et al.*, 2014) and is associated with several functions, including maintaining cortical actin stability during mitosis. Thus, our future directions will involve further functional analysis of Slik and its interaction (direct or indirect) with Sry-a, and its role in regulating actin during cellularization.

685S **Local cell-cell interactions mediate global symmetry breaking in collectively migrating follicle cells** Sierra Schwabach, Sally Horne-BadovinacUniversity of Chicago

The collective migration of cells is a critical process during development, wound healing, and cancer metastasis. We study the highly coordinated collective migration of follicle cells in the Drosophila egg chamber. Follicle cells are arranged in a continuous monolayer around the germ cell cluster. As the newly formed egg chamber buds from the germarium, the follicle cells embark on a circumferential collective migration perpendicular to the anterior-posterior axis, with their basal surfaces crawling on the stationary basement membrane that ensheaths each egg chamber. For the cells to migrate in a directed manner, they must break symmetry and polarize in a uniform direction across the tissue plane. The follicular epithelium provides a unique case study of epithelial planar polarity and migration. The closed topology of the tissue means that there is no external guidance cue, such as free space or a chemical gradient, that dictates the direction of migration. Instead, planar polarity establishment is a stochastic symmetry breaking process that results in clockwise or counterclockwise migration. How symmetry is broken to establish planar polarity is unknown. In addition, it is unclear whether symmetry breaking is a developmentally regulated pre-patterned program, or if it is an emergent self-organized process. This led us to directly test whether there is a discrete developmental window in which migration can initiate. We used the GAL4/UAS system to temporally deplete Fat2, an atypical cadherin continuously required for migration, in follicle cells of the germarium and early stage egg chambers. Strikingly, we found loss of Fat2 before the normal onset of migration does not prevent migration. Instead the start of migration is delayed until Fat2 is expressed at later stages. During this delay, any form of planar polarity that would normally be inherited from the germarium is lost. However, once Fat2 is expressed, tissue polarity recovers with the onset of migration. Together, this suggests that migration initiation is not restricted to a narrow developmental window, nor does it depend on inherited polarity information. This supports a model in which symmetry breaking in the follicular epithelium is a self-organized process that emerges from interactions among the migrating cells themselves.

686S **Rho GTPases Play an Essential Role In Proper Germ Cell Migration** Seohee Ma, Mikayla Gilles, Afshan IsmatBiology, University of St. Thomas

Rho GTPases are known for regulating essential components of cell migration, including cell morphology and actin polymerization. Migration of the primordial germ cells has been studied intensively, however there is still much to learn. The germ cells exhibit round shapes and migrate as a loose collective along the mesoderm from the midgut until they coalesce into two tight clusters on either side of a late-stage embryo. We were interested in looking more closely at the role of Rho1, Rac1, and Cdc42 in germ cell migration. Previous data showed that altering the level of Rho1 activities in germ cells result in germ cell migration defects, as well as survival defects. In this study, both constitutively active (CA) and dominant negative (DN) RhoI, RacI, and Cdc42 were expressed ectopically using the nanos-GAL4 (germ cell specific) driver. In order to visualize cellular protrusions and cell shape changes as a result of migration, we used a myristoylated GFP (myrGFP) as well. Altering levels of Rho1 and Rac1 result in germ cell mis-migration. Specifically, expressing a constitutively active Rac1 results in a tight cluster of germ cells not migrating at all from the midgut. Additionally, expressing a constitutively active Cdc42 results in germ cells stuck to each other and lagging behind during migration with the changes in normal round shapes while others could not even escape from midgut in the earlier stages. Previous studies have suggested that Drosophila E-cadherin (DE-cadherin) plays an important role in enhancing primordial germ cell adhesion. Therefore, we are testing to see if there is a correlation between constitutively active Rac1 or Cdc42 and DE-cadherin. Elucidating the roles of Rho GTPases in germ cell migration, polarization and adhesion will lead to a better understanding of cell migration in general.

687S **The complexities of Fat signaling in the PCP and Hippo pathways** Evan Clark[1], Didier Hodzic[1], Jannette Rusch[1], Heya Zhao[2], Alexey Veraksa[2], Helen McNeill[11]Developmental Biology, Washington University in St. Louis School of Medicine, [2]UMass Boston

Fat (Ft) and Dachsous (Ds) are multifaceted gigantic protocadherins with conserved roles in planar cell polarity, metabolism and growth control. Planar Cell Polarity (PCP) orients cells and tissues into their normal patterns. Ft and Ds are distinct from the core PCP pathway proteins Frizzled, Disheveled, Van Gogh, among others and whether they act

upstream of and/or in parallel to the core PCP pathway has been debated. PCP defects occur when Ft-Ds signaling is disrupted, disordering the normally unidirectional and organized hairs on the wings and proper orientation of ommatidia in the eye.

Fat's intracellular domain functions in tissue growth via the Hippo tumor-suppressor pathway, regulation of which is crucial to developing healthy tissue and avoiding cancer. Fat's role in the Hippo pathway is known to be through key Hippo pathway proteins such as Warts and Expanded, however the biochemical links between Ft and downstream effectors in PCP, growth and metabolism are poorly understood. To obtain better insight, we used CRISPR to place a GFP tag in the intracellular domain of Ft. Affinity purification-mass spectrometry of endogenous Ft-interacting proteins isolated from embryonic lysates was performed by Heya Zhao of the Alexey Veraksa lab. I will present my efforts to validate these interactors and to determine their role in Ft signaling.

688S **Determining the localization of cell-fate determinants in Jagunal-deficient mutant Drosophila embryos** Ethan Lew, Marco Monroy, Blake RiggsBiology, San Francisco State University

Asymmetric cell division (ACD) is fundamental for generating neuronal diversity during development of the Drosophila nervous system. Neuronal stem cells, known as neuroblasts divide asymmetrically to generate self-renewing neuroblasts and a neuronal progenitor cell, a ganglion mother cell (GMC). GMC undergo differentiation to produce neurons and glia cells. Various cell fate determinants regulate ACD in neuroblast cells, including the transcription factor Prospero (Pros). How these cell fate determinants are organized in the cell, delivered to the correct pole and partitioned during mitosis still remains unknown. Previously, our laboratory has identified the highly conserved endoplasmic reticulum (ER) protein Jagunal (Jagn) as necessary for the proper partitioning of the ER. Preliminary data demonstrated that inhibition of Jagn led to a loss of asymmetrical ER partitioning and inheritance. Based on this result, we sought to investigate if Jagn aided in the regulation and partitioning of cell fate determinants during ACD. Here, we used immunocytochemistry to mark specific cell fate determinants during the early stages of Drosophila embryo development. Using both early and late stage Jagn-deficient embryos, we used antibodies against Pros to determine their localization within neuroblast cells during mitosis. Our results suggest that Pros is found to localize as a crescent on the basal membrane of the neuroblast and within the GMC. Future studies will involve determining the localization of Pros in Jagn-deficient neuroblasts, spindle rotation and progress into mitosis of neuroblast cells, and determining the localization of Jagunal with respect to other cell fate determinants in dividing neuroblast cells to determine colocalization and a model of delivery.

689S **Cell polarity opposes Jak-STAT mediated Esg activation that drives intratumor heterogeneity in a *Drosophila* tumor model** Deeptiman Chatterjee[1], Fei Cong[2], Xian-Feng Wang[2], Caique Costa[2], Yi-Chun Huang[2], Wu-Min Deng[21]Biochemistry and Molecular Biology, Tulane School of Medicine, [2]Tulane School of Medicine

In proliferating neoplasms, microenvironment-derived selective pressures promote tumor heterogeneity by imparting diverse capacities for growth, differentiation and invasion. However, compared to the normal epithelia, what makes a tumor cell respond to signaling cues differently is not well understood.In the *Drosophila* ovarian follicle cells, apicobasal-polarity loss induces heterogenous epithelial multilayering. When exacerbated by oncogenic-Notch expression, the multilayer displays an increased consistency in the occurrence of distinguishable cells adjacent to the polar cells.These polar cells release the Jak-STAT ligand Unpaired (Upd), in response to which,the neighboring polarity-deficient cells exhibit a precursor-like transcriptomic state. Using single-cell transcriptomics, we discovered the ectopic activation of the Snail-family transcription factor Escargot (Esg) in these cells. We also characterized the role of Upd and Esg during early follicular development, where the establishment of polarity determines follicle-cell differentiation. Overall, our results establish epithelial-cell polarity as a major gatekeeper against heterogeneity-driving selective pressures from the microenvironment.

690S **CRISPR-mediated investigation of the NaK-ATPase alpha-subunit function in septate junction formation and polarity** Greg J Beitel, Hans AubeeluckMolecular Biosciences, Northwestern University

The NaK ATPase is the well-known essential ion pump that creates the -70 mV gradient across plasma membrane that drives much of cellular solute transport. Less commonly known is that the NaK ATPase also has multiple critical ion transport-independent functions. We previously demonstrated that the "long" isoform of the NaK ATPase alpha subunit, which contains an extra alternatively-spliced 39 amino acids at the N-terminus compared to the "short" isoform, is required for septate junction formation and tracheal tube size control (Paul et al., Development, 2007). Based on results with UAS constructs, the unique septate junction function of the long isoform does not require ion transport. However, the exact function of the long alpha-subunit isoform in septate junction formation is unclear.

To investigate the functions of the long and short alpha-subunits, we have been using CRISPR to mutate individual amino acid and create chromosomes that specifically express the long or the short isoforms. The results obtained with these site-directed mutants will be presented, along with notes on the difficulties of using CRISPR for editing the alpha-subunit locus. While some exons have been straightforward to edit, others have been recalcitrant to CRISPR editing, and others in which CRISPR editing efficiency was typical, Scarless popout efficiency was abnormally poor. For example, Scarless popout efficiencies of in the first long-form exon were less than 0.4% instead of the more typical 20% of progeny, and imprecise excision events were common, which is unusual for a piggybac-based element.

691S **A novel mechanism for transfer of dietary fat to circulating lipoproteins in the larval midgut** Ron Dubreuil, Jasmine HopkinsBiological Sciences, University of Illinois Chicago

We previously described an unusual plasma membrane specialization in the larval fat body of Drosophila. Small cortical lipid droplets (LDs) densely cover the cytoplasmic face of the plasma membrane at sites of lipoprotein docking outside each cell. Knockdown of beta spectrin eliminated the cortical lipid droplet population. We suggested that spectrin organizes a novel lipid uptake apparatus to expedite transfer of non-polar content from lipoproteins outside the cell to LD inside the cell. Here we asked if there is a similar relationship between spectrin and lipid traffic in the midgut. We found that lipid droplets are clustered in a “supranuclear zone” in the anterior midgut, they disappeared after starvation and rapidly reappeared on refeeding. We suggest that lipid droplets are an obligate intermediate in the transfer of dietary fat molecules to lipoproteins that carry fat to the rest of the body. It has been proposed that one carrier (lipid transfer particle; LTP) is endocytosed to gain access to dietary fat. Once loaded, LTP is believed to return to the cell surface for transfer of its cargo to lipophorin (Lpp), the major carrier of dietary fat in the circulation. Expression of an ankyrin1 -GFP reporter in the midgut revealed a stunning array of invaginations of the basal plasma membrane extending all the way up to the supranuclear zone and lipid droplets. The invaginations appear to provide a novel path to bring lipoproteins and lipid droplets into close proximity. Electron microscopy (EM) indicated that the LD and basal invaginations form direct contacts that could facilitate lipid exchange across the plasma membrane. Close contacts between basal invaginations and LD were most frequently observed during refeeding after starvation. Ankyrin depends on beta spectrin, since spectrin knockdown led to mislocalization of ankyrin and dramatic loss of basal invaginations by EM. Interestingly, EM of the beta knockdowns showed that a minor population of invaginations persisted along cell-cell junctions, extending up to the supranuclear region. However, most of the invaginations were reduced to cabbage-like structures at the basal membrane of cells. These data support a model in which spectrin organizes a specialized apparatus to streamline the passage of dietary fat across three boundaries. This mechanism would allow for lipoprotein loading without a need for endocytosis, exocytosis and other complex sorting steps to deliver dietary fat to the blood.

692S **Developing Mass Spectrometry Techniques to Identify Actin Mesh Regulatory Proteins** Merin M RixenChemistry and Biochemistry, UCLA

Actin filaments form networks that play pivotal roles in many cellular processes, including cell polarity establishment. During egg development, oogenesis, an actin network called the actin mesh assists 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 process is highly conserved, 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. Studies have shown that the timely removal of the mesh is integral for the proper establishment of germline polarity. However, little is understood about the mesh and the mechanism behind its 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 bottom-up mass spectrometry to measure protein levels and abundance changes in the *Drosophila* oocyte between mid and late egg development stages. This technique will allow me to identify the proteins that reflect statistically 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. Additionally, this information will promote our 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.

693S **Mapping of a suspected self-interaction domain in β_H-spectrin that may support non-canonical spectrin**

network formation Chelsea Sarring, Claire ThomasBiology, Penn State Univ

Spectrins are large rope-like F-actin crosslinking proteins comprised of two α and two β chains. In the fly, a common α-spectrin subunit forms tetramers with one of two β-spectrin isoforms to create $(\alpha\beta)_2$ and $(\alpha\beta_H)_2$ tetramers. Until recently 2D network formation subtending the plasma membrane was believed to be a critical function of spectrin wherein $(\alpha\beta)_2$ or $(\alpha\beta_H)_2$ tetramers crosslink F-actin filaments to support the membrane and cell structure. However, we previously showed that the specific disruption of spectrin tetramerization, and therefore canonical network formation, by the *α-spec*^{R22S} mutation had no conspicuous effect on *Drosophila* viability and resulted in just a few minor phenotypes. Based on the structure of the $(\alpha\beta_H)_2$-spectrin isoform, it is possible that a self-interaction domain is present in β_H-spectrin that could compensate for the loss of the head-to-head tetramerization interaction in *α-spec*^{R22S} mutants to form an α-spectrin-independent network with F-actin. Such an interaction has been shown to occur in the human homolog of β_H, βV. We report here that the C-terminal region of β_H-spectrin is also self-binding and will present work mapping the location and structure of the minimal binding region within this self-interaction domain.

694S **REC drives recombination to repair double-strand breaks in animal mtDNA** Anna Klucnika[1], Peiqiang Mu[2], Jan Jezek[1], Matthew McCormack[1], Ying Di[1], Charles Bradshaw[1], Hansong Ma[11]University of Cambridge Gurdon Institute, [2]South China Agricultural University

Mechanisms that safeguard mitochondrial DNA (mtDNA) limit the accumulation of mutations linked to mitochondrial and age-related diseases. Yet, pathways that repair double-strand breaks (DSBs) in animal mitochondria are poorly understood. By performing a candidate screen for mtDNA repair proteins, we identify that REC — an MCM helicase that drives meiotic recombination in the nucleus — also localizes to mitochondria in *Drosophila*. We show that REC repairs mtDNA DSBs by homologous recombination in somatic and germline tissues. Moreover, REC prevents age-associated mtDNA mutations. We further show that MCM8, the human ortholog of REC, also localizes to mitochondria and limits the accumulation of mtDNA mutations. This study provides mechanistic insight into animal mtDNA recombination and demonstrates its importance in safeguarding mtDNA during ageing and evolution.

695S **Rattling the chains of a ghost protein: spectrin** Zane Deliu, Ronald DubreuilBiological Sciences, University of Illinois at Chicago

Much has been learned from studies of the protein spectrin in Drosophila. We now know a lot about its activities and phenotypes of numerous mutants that have been studied. Roles have been implicated in cell shape and interactions, neuronal function, axon guidance, dietary fat processing, planar polarity, and more recently has been implicated in the Drosophila Parkinson's model as an agent of the phenotype caused by high expression of mammalian alpha - synuclein. Many questions about the assembly and function of spectrin are still unanswered, and consequently Drosophila remains a powerful system for further exploration. Most of the known functional sites in the molecule are associated with the beta subunit. Here we present a progress report on issues we have examined. First, we engineered mutations intended to eliminate actin binding activity. Two alleles lacked function in vivo, but because both proteins were unstable it was not possible to assess whether they lost actin binding activity. A third allele was functional in vivo but produced a striking effect on fly size (Kpn+3). Here we assessed its actin binding activity and found that it was comparable to wild type. Thus, we have yet to generate an actin binding defect. Second, we demonstrated by transgene rescue that spectrin is essential in neurons, but not in most of the other tissues in which it is expressed. Elav gal4 driven UAS Beta transgene expression overcomes the lethality of a functional null mutation. Here we found that with elav-driven expression of RNAi (Trp) there was a striking lethality of both elav and synaptobrevin Gal4 (BL51945) - driven RNAi for beta spectrin (BL 42802). The lethality appears to be on-target since co-expression of wild type UAS Beta spectrin transgene partially reversed the lethality of RNAi. Third, we used gal80 ts to assess the lethal phase of loss of beta spectrin function. Lethality was consistently observed with knockdown in the first few hours of development, but rapidly dropped off when knockdown was initiated at later stages on through adulthood. Fourth, we produced a recombinant chromosome carrying both alpha and beta spectrin UAS transgenes. We find that Beta spectrin over expression alone is usually toxic, but toxicity is ameliorated by co-expression of alpha spectrin. Elevating expression of both subunits simultaneously leads to higher net level of expression than when either subunit is expressed alone.

696V **Role of conserved molecular scaffold Tudor complex in the assembly of membraneless organelles in Drosophila germ cells** Samuel J Tindell, Wahiduzzaman Wahiduzzaman, Ethan Hackney, Emma Alexander, Ryan Schmidtke, Alexey L ArkovMurray State University

Similar to other animals, Drosophila germ cells assemble landmark membraneless RNA-protein organelles referred

to as germ granules. These granules form at the posterior of the developing oocyte and, after the egg is laid, they are maintained in the embryo's posterior before germ cell formation. One of the principal protein components of germ granules is a scaffold protein Tudor, which contains 11 evolutionarily conserved protein-protein interaction modules called Tudor domains. Tudor and its binding partner proteins, including Piwi protein Aubergine, an ATP-dependent RNA helicase and ATP-producing enzyme Pyruvate Kinase, are required for the assembly of germ granules and germ cell development. Using systematic molecular and super-resolution imaging analysis, we are aiming at providing molecular understanding of how Tudor and its binding partners are contributing to the assembly of germ granules. In particular, we are comprehensively dissecting the assembly mechanisms of Tudor binding partners to all 11 Tudor domains individually and in the context of larger Tudor polypeptide. Unexpectedly, our data show that Aubergine and Pyruvate Kinase use different modes to interact with Tudor domains of Tudor scaffold. In addition, our super-resolution microscopy data are consistent with this molecular analysis and further suggest that during germ granule assembly, different protein components of the granules are localized to discrete partially overlapping regions. Our work provides mechanistic insights into the assembly of membraneless organelles in Drosophila germline.

697V **E2 ubiquitin conjugase Bendless is essential for PINK1 stability and PINK1-Park mediated degradation of Marf** Rajit Narayanan Cheramangalam[1], Tarana Anand[1], Priyanka Pandey[2], Deepa Balasubramanian[1], Reshmi Varghese[2], Neha Singhal[1], Sonal Nagarkar Jaiswal[2], Manish Jaiswal[11]Tata Institute of Fundamental Research, Hyderabad, [2]CSIR–Centre For Cellular And Molecular Biology

Mitochondria undergo dynamic changes in their size in response to metabolic changes and to maintain mitochondrial quality. The change in mitochondrial size is mediated by organelle fission and fusion. In this work we sought to identify regulators of Mitofusin/Marf, which is required for mitochondrial outer membrane fusion. Through an unbiased genetic screen we identified mutations in lrpprc2 that resulted in reduction in Marf levels. LRPPRC is required for mRNA stability and its loss induces mitochondrial stress. We show that the reduction in Marf levels in lrpprc2 mutants is caused by proteasomal degradation through E3 ubiquitin ligase Park and its activating kinase PINK1. PINK1-Park are often associated with mitophagy, the selective clearance of damaged mitochondria, however in lrpprc2 mutants we neither find mitophagy nor the presence of any previously known triggers of PINK1-Park activation such as loss of mitochondrial potential or increased ROS. Further, we found a K63 specific E2 ubiquitin conjugating enzyme Bendless is also required for Marf degradation in lrpprc2 mutants. We found that Bendless is essential for PINK1 stability and hence it is important for PINK1 mediated Marf degradation. Further, we observed hyperfused mitochondrial phenotypes in lrrprc2 bendless double mutants as compared to lrpprc2 or bendless mutants, suggesting that Bendless is required for mitochondrial size regulation. Moreover, we observed severe eye degeneration in lrpprc2 bendless double mutants, suggesting a neuroprotective role of Marf degradation in lrpprc2 mutants. In conclusion, the increased Marf turnover by Bendless-PINK1-Park under mitochondrial stress helps in cellular survival, probably by segregating damaged mitochondria.

698V **Single-cell transcriptomics identifies Keap1-Nrf2 regulated collective invasion in a Drosophila tumor model** Deeptiman Chatterjee[1], Caique Costa[2], Caique Costa[3], Xian-Feng Wang[1], Allison Jevitt[4], Yi-Chun Huang[1], Wu-min Deng[11]Biochemistry and Molecular Biology, Tulane University, [2]Tulane University, [3]Biochemistry and Molecular Biology, Tulane University School of Medicine, [4]Cancer and Cell Biology Department, Oklahoma Medical Research Foundation

Apicobasal cell-polarity loss is a founding event in Epithelial-Mesenchymal Transition (EMT) and epithelial tumorigenesis, yet how pathological polarity loss links to plasticity remains largely unknown. To understand the mechanisms and mediators regulating plasticity upon polarity loss, we performed single-cell RNA sequencing of *Drosophila* ovaries, where inducing polarity-gene *l(2)gl*-knockdown (Lgl-KD) causes invasive multilayering of the follicular epithelia. Analyzing the integrated Lgl-KD and *wildtype* transcriptomes, we discovered the cells specific to the various discernible phenotypes and characterized the underlying gene expression. A genetic requirement of Keap1-Nrf2 signaling in promoting multilayer formation of Lgl-KD cells was further identified. Ectopic expression of Keap1 increased the volume of delaminated follicle cells that showed enhanced invasive behavior with significant changes to the cytoskeleton. Overall, our findings describe the comprehensive transcriptome of cells within the follicle-cell tumor model at the single-cell resolution and identify a previously unappreciated link between Keap1-Nrf2 signaling and cell plasticity at early tumorigenesis.

699V **Phosphatidylserine maintains mitochondrial homeostasis through coupling Ca^{2+} relay with lipid metabolism** Yifan ZhouInstitute of Genetics and Developmental Biology, Chinese Academy of Sciences

Orchestrated phospholipid composition is essential for mitochondrial homeostasis. Phosphatidylserine (PS) has been proved important for mitochondria, while established knowledge restricted this importance to the role of PS in

producing phosphatidylethanolamine (PE) inside the mitochondria. Here, we report that PS maintains mitochondrial homeostasis through governing the PE composition in the endoplasmic reticulum (ER), which is tightly associated with SREBP signaling in *Drosophila.* Based on a large-scale suppressor screen, Ca^{2+} overload was identified as the direct cause for mitochondrial defects on the context of disturbed phospholipid environment when intracellular PS reduced. Mechanically, in response to intracellular PS deficiency, altered PE composition enhanced ER-to-mitochondrial Ca^{2+} transport through inositol trisphosphate receptor (*itpr*). Either rebalancing lipid environment or inhibiting ER-to-mitochondrial Ca^{2+} transport is sufficient to suppress the mitochondrial perturbations. In addition, JNK was also activated by reduced intracellular PS content, and targeted on the *itpr* in *Drosophila*. We demonstrated that JNK-Ca^{2+} axis served as a conserved strategy for suppressing mitochondrial defects in both the PS-depleted fly tissue and HeLa cells, unveiling new insights for mitochondrial diseases derived from phospholipid perturbations.

700V **Myc regulates Stress-Induced Mitochondrial Biogenesis in *Drosophila*** Aravind H, Sonia Sandhi, Deepa Balasubramanian, Aishwarya Shishir Mandya, Mrunal Nagraj Kulkarni, Tanya Singh, Manish JaiswalTIFR Hyderabad

Mitochondrial content is dynamically regulated in cells in order to adapt to its various physiological, developmental, and metabolic requirements. The pathways that regulate this dynamicity in mitochondrial content by sensing and responding to cellular cues are still incompletely understood. To identify novel regulators of mitochondrial content, we performed a forward genetic screen in *Drosophila* and identified mutations in 15 nuclear genes encoding mitochondrial proteins that lead to an increase in mitochondrial content. A similar increase in mitochondrial content has been previously seen in mouse respiratory chain deficient mutants as well as in mitochondrial diseases like MELAS. However, the mechanisms that govern this response and its consequences are not understood. We term this phenomenon Stress-Induced Mitochondrial Biogenesis (SIMB). To investigate regulators of SIMB, we used *lrpprc2* mutants as a model system. We show that SIMB is regulated in a Myc-dependent and PGC1α-independent manner. Furthermore, transcriptomics of *lrpprc2* mutants showed that several myc-targeted genes are upregulated. Put together, our results reveal a hitherto unknown role for Myc in regulating mitochondrial biogenesis during mitochondrial dysfunction.

701V **Genetic Interaction between Ribonucleoprotein Clueless and Mitochondrial Permeability Transition Pore Components and Regulators** Hye Jin Hwang[1,2], Rachel Cox[11]Uniformed Services University of the Health Sciences, [2]Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

The mitochondrial permeability transition pore (MPTP) is an unselective voltage-dependent channel embedded in the mitochondrial inner membrane. Stimuli such as calcium overload, uncoupled oxidative phosphorylation, increased reactive oxygen species, and increased free fatty acids have been shown to open MPTP. Prolonged irreversible opening of MPTP causes mitochondrial matrix solutes up to 1500 Da to non-selectively flood the cytosol, which accompanies an influx of water into the mitochondrion. This results in mitochondrial swelling, disruption of ion homeostasis, and cytochrome c release, consequently inducing cell death and pathological effects such as ischemia/reperfusion, muscle dystrophy, and neurodegeneration. Transient reversible opening of MPTP also occurs and may be involved in calcium homeostasis and mitochondrial remodeling. Though MPTP components have not been unambiguously identified, a promising candidate is an F-ATP synthase working with several regulators such as VDAC (Porin), ANT (SesB), cyclophilin D, and OSCP (ATPsynO). We hypothesize that the highly conserved ribonucleoprotein, Clueless (Clu), may interact with MPTP in Drosophila. Clu is required for physiological functions such as energy production, metabolic pathways, mitochondrial quality control, and stress responses in various species. In addition, Clu binds to cytosolic translational machinery for mitochondrial protein synthesis. *Drosophila* Clu forms highly dynamic cytoplasmic particles depending on the cellular nutrient state or stress environments. Interestingly, *clu* genetically interacts with *pink1* and *parkin*, which respond to mitochondrial membrane depolarization and induce mitophagy. Several studies by others have also shown that Clu is involved in mitophagy induced by mitochondrial membrane depolarization in other species. In addition, Clu physically interacts with the proteins in mitochondrial outer membranes, such as Porin and TOM20. Considering all of these, we examined whether Clu may be functionally related to MPTP. We have found that *clu* genetically interacts with components of MPTP, causing mitochondrial morphological changes. We will present data to determine whether this causes mitochondrial dysfunction as well. Because the role of Clu in response to cellular stress environments, including oxidative stress, has not been clear yet, this study›s observation will elucidate how Clu participates in mitochondrial stress response and maintenance of mitochondrial structures.

702V **Dual model organism analysis identifies shared and unique features of tissue hingepoint formation** Juana De La O[1], Chidera Okeke[1], Adam Martin[21]Biology, Massachusetts Institute of Technology, [2]Massachusetts Institute of Technology

Animal tissues are sculpted into diverse shapes during embryonic development. However, different animal tissues exhibit similar shape changes, like the bending of a flat tissue to generate a hingepoint. One such hingepoint forms in the center of the bending mesoderm of gastrulating *Drosophila* embryos. We previously showed that *Drosophila* mesoderm hingepoint formation - 1) is associated with a multicellular gradient of myosin-II, and 2) requires cytoskeletal dynamics to propagate forces. One regulator of cytoskeletal dynamics is cytoplasmic Calcium, which is stored in the cell's endomembrane system. To test whether other bending tissues require features present in *Drosophila* gastrulation, we analyzed the distribution of cytoskeletal components during mouse neural plate bending. Here, we show the mouse neural plate exhibits dynamically changing myosin-II patterns during bending. Additionally, these patterns are disrupted in neural plates with a mutant secretory pathway calcium ATPase 1 (SPCA1). The mechanism by which this conserved calcium pump regulates the cytoskeleton in bending tissues is unclear. Therefore, we maternally knocked down the SPCA1 orthologue, SPoCk in *Drosophila* embryos. SPoCk depleted embryos exhibited abnormal gastrulation movements, including during mesoderm bending. Taken together, our results 1) demonstrate that morphological processes as diverse as *Drosophila* mesoderm invagination and mouse neural tube closure can be governed by similar mechanical processes, 2) suggest a conserved role for SPCA1/SPoCk in tissue morphogenesis, and 3) highlight the utility and power of cross-organism studies. Our future work aims for a finer-grain analysis of cytoskeletal dynamics in live mouse embryos to facilitate comparisons with gastrulating Drosophila embryos as well as further interrogating the molecular relationship between SPCA1/SPoCk mediated calcium dynamics and the cytoskeleton-mediated bending process.

703V **Cryo-EM structure of mitochondrial complex I from *Drosophila melanogaster*** Abhilash Padavannil[1], Anjaneyulu Murari[2], Shauna-Kay Rhooms[2], Edward Owusu-Ansah[2], James Letts[11]University of California, Davis, [2]Columbia University Irving Medical Center

Mitochondrial complex I (CI) is a proton-pumping oxidoreductase in the inner mitochondrial membrane (IMM) crucial to bioenergetic metabolism. CI is composed of more than 40 distinct subunits. CI subunits can be divided into a set of 14 highly conserved central or core subunits and a variable number of accessory subunits between different organisms. CI couples the transfer of electrons from NADH to CoQ to the pumping of four H^+ ions across the IMM. Structures of CI from all organisms assessed to date reveal a boot-shaped structure consisting of two arms: a peripheral arm that extends into the mitochondrial matrix and a membrane arm that is embedded in the IMM. Here, we describe the structure of mitochondrial CI from the thoracic muscles of *Drosophila melanogaster*. We find that 29 of the 30 mammalian CI accessory subunits are conserved in fruit flies. Further, even among conserved subunits, some aspects of the structure are distinct from mammals, providing insight into the role of mammalian subunits by comparison. Although *Drosophila melanogaster* complex I (*Dm*-CI) does not deactivate, the resting state of *Dm*-CI adopts multiple conformations. We also identify a helix-locked open state in which an N-terminal alpha helix on the NDUFS4 subunit wedges between the peripheral and membrane arms, as well as several other features of the complex that differ from conformational states observed in bacteria, yeast and mammals. Given the extensive homology between *D. melanogaster* and *D. suzukii* – a major pest for soft summer fruits – we postulate that *Drosophila*-specific features of CI may uncover novel regulatory mechanisms of the complex that can be exploited to generate new pesticides for *D. suzukii*.

704T **Systemic propagation of cytosolic DNA response via generation of large extracellular vesicles** Jiae Lee, Hyungjoon Park, Young KwonBiochemistry, University of Washington

Tumor-secreted extracellular vesicles are emerging as critical mediators of intercellular/interorgan communication between tumor cells and stromal cells in local and distant microenvironments. Although the physiological roles of secreted extracellular vesicles (EVs) are demonstrated from embryo development to immune system stimulation, most of the previous studies have been performed in vitro. Utilizing a metastatic tumor model in the *Drosophila* larval eye disc ($Ras^{V12}/scribble^{-/-}$) to readily observe, collect, and manipulate the tumor EVs, we have developed an effective system to isolate tumor derived EVs and validated the accumulation and intactness of the EVs. Using this system, we identified the cytosolic DNA-sensing cGAS-STING pathway as the key mechanism of EV formation in the metastatic tumor. cGAS is an innate immune sensor that recognizes a diverse array of cytosolic dsDNA that cancer cells are often filled with. Activated by cGAS, STING mediates the transcriptional activity of a broad repertoire of molecular programs, which include inflammation, senescence, autophagy, and metastasis. Next, we established a novel EV injection model as an in vivo platform to test the function of tumor EVs in the host tissue. Tumor EVs activate the host immune system by its interaction with hemocytes, the macrophage-like immune cells in *Drosophila*. Intriguingly, tumor EVs are engulfed by the hemocytes, and ablation experiments suggest hemocytes relay tumor EV-induced immune responses and STING signaling is required in the hemocytes for this process. Moreover, we confirmed that cGAS is present in the large EVs produced from the mammalian cancer cell lines, indicating a role of EVs as systemic propagation of cytosolic DNA response to the

host tissue. Altogether, this study elucidates the molecular mechanisms of tumor EV formation and its action on the host tissue that may modulate tumor immunogenicity or pre-metastatic conditioning.

705T **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 A. Nasr[1], Jeff Sekelsky[2,31]Curriculum in Genetics and Molecular Biology, The University of North Carolina at Chapel Hill, [2]Department of Biology, The University of North Carolina at Chapel Hill, [3]Integrative Program for Biological and Genome Sciences, The University of North Carolina at Chapel Hill

DNA gap repair is essential for protecting against genomic instability, a hallmark of cancer. Normally, DNA gaps refer to ssDNA gaps. Here, we use DNA gaps to refer to dsDNA gaps. These gaps can be the outcome of different events such as transposable element excision or closely spaced DSBs. Revealing gap repair mechanisms is not only important for our understanding of genome integrity maintenance, but it is also important for optimizing different biological applications such as Cas9 gene drive and CRISPR/Cas9 genome editing. Different aspects of gap repair require more investigation, including strand invasion dynamics. During invasion of the template strand, a D loop is formed. Two models have been hypothesized to explain D-loop dynamics: an extended D-loop model and a migrating D-loop model. Yet, evidence is lacking for both models. Also, it has been hypothesized that gap repair involves several rounds of resection, strand invasion, and synthesis. We aim to develop a gap repair assay in Drosophila to fill this knowledge gap. We will use the CRISPR/Cas9 system to insert a target sequence and a template sequence on the third chromosome, with each on only one of the homologous pairs. We will use 4 different templates representing 4 different gap lengths: 0, 4, 8, and 12 kb. After that, CRISPR/Cas9 system will use a gRNA in the target locus to induce a DSB within that target. We will detect single-stranded DNA through APOBEC-mediated mutagenesis. Human APOBEC3A deaminates cytidine into uridine in ssDNA. In the extended D loop model, the displaced template strand is single-stranded while in the migrating D loop model, the nascent strand is single-stranded. Using long-read sequencing, we expect C-to-T mutations in the template sequence in the case of an extended D-loop model and G-to-A mutations in the case of a migrating D-loop model. Furthermore, mutations in sequences flanking the target sequence would indicate the extent of resection. Flies express SMUG1, an enzyme that removes uracil from ssDNA. Therefore, we will use the CRISPR/Cas9 system to disrupt *SMUG1*. Finally, we hypothesize that loss of Bloom helicase activity in Bloom mutants would result in an extended D loop, exhibiting the G to A mutational signature. This gap repair assay will help understand the details of the gap repair process and aid will further have an impact on optimizing gap repair-dependent biological applications.

706T **Molecular determinants of Crumbs overexpression induced neoplastic tissue growth** Maksym Shcherbina, Parama Talukder, Ulrich TepassDepartment of Cell and Systems Biology, University of Toronto

Crumbs (Crb) is an apical transmembrane protein that regulates epithelial polarity and can modulate tissue growth through interactions with the Hippo and JNK pathways. Both mammalian and Drosophila Crb proteins have been linked to the regulation of tissue growth and tumor development. Crb overexpression in fly imaginal disc epithelia causes neoplastic overgrowth (Lu and Bilder, NCB, 2005). Here we asked which factors are required for neoplastic tumorigenesis when Crb is overexpressed.

Moderate overexpression of Crb activates the Hippo pathway effector Yorkie and the JNK pathway to stimulate over proliferation and neoplastic transformation. Surprisingly, high level overexpression of Crb switches JNK from acting pro-proliferatively to having proapoptotic effects, reducing tumor size. Reducing JNK activity in this context restores full neoplastic development suggesting that high-level Crb overexpression further activates JNK which overrides the pro-proliferative mechanisms downstream of Crb. In addition to Yorkie and JNK, we are particularly interested in determining which factors of the epithelial polarity machinery are required for Crb-inducted neoplastic development. We first tested several Crb constructs with modified cytoplasmic domains. We found that the loss of either the C-terminal PDZ domain or the juxtamembrane FERM domain reduced the Crb-induced neoplastic phenotype. This suggests that bindings partners of Crb that interact with the FERM domain binding site and the PDZ domain binding site are required for neoplastic growth. We are currently testing known Crb binding partners and other components of the epithelial polarity machinery for their requirement in Crb-induced neoplastic growth.

707T **Neuroendocrine regulation of cell competition** Jeffrey L Bellah[1], Cora Bergantiños[1], Albana Kodra[1], Abigail Svoysky[2], Laura Johnston[11]Genetics and Development, Columbia University Medical Center, [2]Chemistry and Chemical Biology, Harvard University

Cell competition results from cellular interactions in which normally viable, but less “fit”, cells are eliminated from a

growing tissue due to their interactions with relatively more fit cells in the tissue. This bears a marked resemblance to the phenomena of field cancerization, in which pre-neoplastic cells spread within a tissue and can be a source of cancer recurrence following tumor resection. We have developed tools to study Myc super-competition (MSC), a competitive context wherein cells with increased levels of the growth factor and oncogene Myc compete with wild-type cells in wing imaginal discs of young *Drosophila* larvae. Although cell competition occurs via local interactions between wing disc cells, we find that it is also influenced by circulating hormones. Our data indicate that MSC requires a neuroendocrine axis that consists of the secreted *Drosophila* insulin-like peptide 8 (Dilp8) and its receptor, leucine-rich repeat-containing G-protein coupled receptor 3 (Lgr3). Dilp8 is made in and secreted by wing discs and other larval tissues, but *lgr3* expression is restricted to specific neurons in the CNS and brain. At the end of larval development, Dilp8/Lgr3 activity modulates synthesis of the steroid hormone Ecdysone in the prothoracic gland, the major larval endocrine organ, thereby helping to coordinate wing disc growth with overall larval development. We find that in *dilp8* mutants, overall larval Ecdysone levels are aberrantly enhanced during the rapid growth phase of the early 3rd instar, and are associated with a block to MSC. These findings suggest that Dilp8/Lgr3 activity normally promotes competitive intercellular interactions during the growth phase by limiting the circulating level of Ecdysone. We will discuss our efforts to determine how Dilp8/Lgr3 activity promotes MSC, and whether it relates to Ecdysone signaling activity in the wing disc or other larval tissues. Understanding the mechanism by which systemic control of hormone levels influences competitive cell-cell interactions in remote peripheral tissues may aid in the development of therapeutic approaches to target field cancerization.

708T **A genetic screen and characterization of triggers of cell competition.** Hiroshi Kanda, Ryo Matsumoto, Aya Kawasaki, Mai Nakamura, Rina Nagata, Xinyue Huang, Ayumu Okumura, Naotaka Ochi, Yoji Noguchi, Naoki Wakasa, Ryosuke Nakano, Tatsushi IgakiKyoto University

Cell competition is a context-dependent cell elimination whereby cells with higher fitness eliminate neighboring intrinsically viable, less-fit cells by inducing apoptosis. We have conducted a large-scale EMS-based genetic screen for mutations that trigger cell competition in Drosophila eye discs. As a result, we isolated 65 cell-competition trigger mutations from 12,496 mutagenized chromosomes. Genetic characterization of these mutations revealed that 87.7% (57/65) of them induce cell competition that depend on b-Zip transcription factor Xrp1, JNK, or both. This result illuminates the prospect that (1) a variety of physiological/pathological stimuli could trigger cell competition, and (2) a particular pathway(s) could comprise a core machinery of cell competition. We further found by examining mutations on 3R chromosome arm that 8 mutations (29.6%) induce cell competition that depend on ATG1, an essential regulator of autophagy. All these eight mutations induced cell competition in a Xrp1-dependent manner. These analyses enabled us not only to identify the “canonical pathways” of cell competition but to conduct further modifier screens. We are currently mapping the responsible genes for mutations and investigating the molecular mechanisms of cell competition triggered by different mutations, which will be presented.

709T **The impact of cell cycle and sex on DNA double-strand break repair** Elizabeth Graham, Jan LaRocqueHuman Science, Georgetown University

Accurate repair of DNA double-strand breaks (DSBs) is essential for the maintenance of genome integrity, as failure to repair DSBs results in cell death. The cell has evolved three main mechanisms for DSB repair: non-homologous end-joining (NHEJ), single-strand annealing (SSA) and homologous recombination (HR). While certain factors like age and state of the chromatin are known to influence DSB repair pathway choice, the role of cell cycle and sex on pathway choice has yet to be elucidated in multicellular organisms. To examine the influence of cell cycle, we analyzed DSB repair in male larval (cycling) and adult (non-cycling) brains. DSB repair was evaluated by molecular analysis of the DR-*white* assay using the TIDE method. In the larval brains, 80.4% of DSBs were repaired by HR, while only 28.1% of breaks in adult brains were repaired by HR ($p = 2.1 \times 10^{-31}$). Analysis of female brains revealed no significant differences compared to the males, but the influence of cell cycle on pathway choice in females remained clear ($p = 2.1 \times 10^{-31}$). Furthermore, when examining the whole fly (somatic and germline), females repaired 48.1% of breaks by HR (51.9% by NHEJ), similar to males, who repaired 47.3% by HR and 52.8% by NHEJ ($p > 0.05$). We also investigated the mitotically-dividing premeiotic germline for sex-specific differences in pathway choice. Using phenotypic analysis of the DR-*white* assay, we identified a statistically significant decrease in homology-directed repair (HR: $p = 5.2 \times 10^{-19}$; SSA: $p = 1.49 \times 10^{-9}$) in female premeiotic germlines compared to the males. To understand the mechanism behind this difference, we will determine whether the decrease in HR in the female germline is due to p53-dependent apoptosis. This analysis will occur by examining repair in the males and females in a *p53* mutant background both molecularly in whole flies and phenotypically in the pre-meiotic germline.

710T **A spatiotemporal cell cycle model of the mid-blastula transition** Yuki Shindo, Amanda AmodeoDepartment of Biological Sciences, Dartmouth College

Proper development requires that cellular events occur in the correct order and at the correct time. However, underlying principles for timing regulation in development remain unclear. We use the mid-blastula transition (MBT) in the *Drosophila* embryo as a model to study the fundamental mechanisms that underlie the control of timing in development. The early embryos of many metazoans spend the first several hours post-fertilization rapidly dividing their large reservoirs of cytoplasm into thousands of smaller cells and nuclei. After a species-specific number of divisions, the embryos switch from rapid and synchronous divisions to slower and patterned divisions at the MBT. We have recently found that excess histone H3 acts as a signaling molecule that promotes cell cycle progression and is critical for timing the MBT. However, it remains unclear how the activity of the cell cycle dynamically changes during embryogenesis to coordinate developmental progression with cell cycle remodeling at the MBT. Here, we hypothesize that subcellular dynamics of the cell cycle regulators underlie the robust timing regulation of the MBT. Using live imaging, we show that rates of H3 nuclear import decrease with developmental progression, resulting in a reduction in nuclear H3 concentrations. By contrast, rates of nuclear import remain relatively unchanged for other substrates including a key cell cycle regulator Chk1 and a nuclear marker NLS-RFP. Using mathematical modeling, we show that these distinct regimes for nuclear import result in dynamic changes in the nuclear composition and lengthening of cell cycle durations during cleavage divisions. Our model also finds that the H3-specific nuclear import regime contributes to the robustness of cell cycle remodeling. To understand the mechanistic underpinnings, we focus on the nuclear pore, which is the sole gateway for macromolecular shuttling between the nucleus and the cytoplasm. We create an endogenously-tagged marker for the nuclear pore and find that the nuclear pore numbers decrease with developmental progression. This reduction in the nuclear pore numbers corresponds to the decrease in bulk nuclear import and cell cycle slowing at the MBT. Additionally, we show that depletion of nuclear pores globally delays development, suggesting that the nuclear pore sets the timescale of early embryogenesis. Together, we propose that the dynamics of nucleocytoplasmic shuttling govern precise temporal control of early development.

711T **Novel Upstream Regulation of Actomyosin-mediated Growth Control** Liang Hu[1], Wyatt Brichalli[2], Naren Li[1], Shifan Chen[3], Yaqing Cheng[1], Qinfang Liu[3], Yulan Xiong[3], Jianzhong Yu[41]Physiology and Neurobiology, University of Connecticut, [2]Anatomy & Physiology, Kansas State University, [3]Department of Neuroscience, University of Connecticut School of Medicine, [4]University of Connecticut

The actomyosin cytoskeleton has been suggested to play an important role in mediating the Hippo signaling pathway, an evolutionarily conserved developmental pathway that controls organ size and tissue homeostasis from *Drosophila* to mammals. Despite the established function of the actomyosin cytoskeleton in the Hippo pathway, the upstream regulation of actomyosin-mediated growth control is less defined. In this study, we identified phosphoinositide-3-phosphatase Myotubularin (Mtm) as a novel upstream regulator of actomyosin that functions synergistically with the Hippo pathway in growth control. Our study suggests that Mtm regulates three major cellular functions including F-actin dynamics, membrane protein distribution, and actomyosin activation. We further demonstrated that actomyosin functions as the most downstream effector of Mtm in growth control. Mechanistically, Mtm regulates membrane phospholipid PI(3)P dynamics, which in turn modulates actomyosin activity through Rab11-mediated vesicular trafficking. Our study also shows that MTMR2, the human counterpart of *Drosophila* Mtm, has conserved function in regulating actomyosin activity and tissue growth. Taken together, our study suggests a critical role of the membrane lipid PI(3)P in regulation of actomyosin activity and provides a novel functional link between membrane lipid dynamics and growth control. Our study also sheds new insights into the molecular basis of MTMR2-related peripheral nerve myelination and human disorders.

712T **Deficiency of Blm DNA helicase during early embryonic cell cycles establishes detrimental outcomes in surviving progeny** Brayden Graves, Kyra Lockett, Kenedi Freeman, Abygail Marler, Sara Hathaway, Eric P StoffregenPhysical, Life, Movement & Sport Sciences, Lewis-Clark State College

Blm DNA helicase is essential for ensuring proper DNA replication and genome integrity during the earliest cell divisions in *Drosophila* development. These cell divisions rely on maternally provided Blm products until zygotic products take over functional control. As a result, *Blm* females, who are unable to provide functional Blm products to their eggs, display a severe maternal effect lethality. Embryos from *Blm* mothers accumulate DNA damage during embryo development, and nearly all die prior to hatching. We hypothesized that the small proportion of progeny that survive this Blm-deficient development would experience sub-lethal DNA damage that affects measures of lifespan and healthspan. To test these

hypotheses, adult flies that developed with or without maternal Blm, but were otherwise genetically identical, were compared. We found that Blm-deficient development led to a reduction in lifespan, increased neurodegeneration, defects in metabolic function, and changes to circadian patterns. These data indicate that early exposure to DNA damage caused by Blm-deficiency has consequences long after the restoration of Blm expression.

713T **Genetic interactions between Headcase and insulin signaling in growth control** Sam Simonovitch[1], Naren Li[1], Qinfang Liu[2], Yulan Xiong[2], Jianzhong Yu[11]Department of Physiology and Neurobiology, University of Connecticut, [2]Department of Neuroscience, University of Connecticut School of Medicine

Nutrient restriction (NR) reduces the incidence and growth progression of many types of tumors, yet its underlying mechanism is not well understood. We recently identified Headcase (Hdc) as a tumor suppressor that regulates tissue growth in response to NR. While identifying Hdc as an NR-specific growth regulator is intriguing, questions remain about the underlying regulatory mechanisms. Specifically, the upstream signal that makes Hdc an NR-specific growth regulator needs to be defined. The insulin signaling network is critical in controlling cell growth, proliferation, and metabolism in response to diverse upstream inputs such as growth factors, nutrients, oxygen, and energy levels. We reveal strong genetic interactions between Hdc and multiple Insulin signaling pathway components, demonstrating that the growth inhibition function of Hdc is negatively regulated by Insulin signaling. We also show that Hdc physically interacts with Akt, a major kinase in insulin signaling. Taken together, these findings suggest that insulin signaling may link nutrition status to Hdc growth control function.

714T **Investigating the role of Uif in tissue-specific growth of the larval trachea** Zihao Yu[1], Robert Ward[21]Case Western Reserve University, [2]Biology, 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 mechanisms of tissue-specific grow is important because severe defects in these processes can lead to inviable embryos, and subtle differences in tissue-specific growth are associated with traits important for evolution and speciation. The Drosophila larval trachea is an excellent model system since it shows tissue-specific growth and can be easily visualized due to the optical properties of the air/liquid interface in the trachea. In addition, we and other labs have identified genes that are required for growth specifically in the larval trachea. One such gene is *uninflatable* (*uif*). *uif* encodes a single pass transmembrane protein that is expressed on the apical surface in epithelial cells, and is strongly expressed in the trachea. Loss of *uif* results in larval lethality with trachea that are roughly half the relative size of that found in wild type larvae. Preliminary evidence using a 3rd chromosome BAC that includes the entire *uif* genomic locus indicates that 3 copied of *uif* leads to larval tracheal overgrowth. In addition, we recovered mutations in *PNPase* that lead to overgrown trachea, and these mutant trachea also express significantly higher levels of Uif protein. Together, these results suggest a positive relationship between Uif expression and trachea length. To investigate the stochiometric relationship between Uif protein level and tracheal growth, we are measuring trachea length from 3rd instar larvae carrying 0, 1, 2 or 3 copy of *uif*, and quantifying Uif protein levels using a semi-quantitative confocal approach. The larval trachea is an endoreplicating tissue whose growth is controlled through the insulin signaling pathway. We are examining readouts of the insulin signaling pathway in *uif* and other tracheal-specific growth mutants to determine whether and where these genes act within this pathway.

715T **Spc105R is required for kinetochore assembly, cohesion protection, and regulating modes of microtubule interactions in Drosophila oocytes.** Lia Mahal, Kim McKimGenetics, Rutgers University

Research on meiosis is aimed at understanding how genetic material is accurately passed from parent to offspring and discerning causes of infertility, miscarriages, and genetic disorders. The kinetochore protein SPC105R, the *Drosophila* homolog for KNL1 in humans, is an essential part of the meiotic pathway. Some important functions of SPC105R include the assembly of the kinetochore, establishing kinetochore-microtubule attachments, and interacting with regulatory proteins such as the MPS1 and Aurora B kinases. SPC105R has several different domains that have specific roles in these functions. The C-terminal domain is thought to build the kinetochore and is responsible for kinetochore-microtubule attachments, and the other domains regulate attachments to ensure errors do not occur. Errors in attachments occur when both kinetochores are attached to the same pole (mono-oriented). Biorientation of the kinetochores to opposite poles is crucial for proper chromosome segregation. The N terminal region (NTR) of SPC105R has two conserved Aurora B phosphorylation sites which are suspected to be important for the regulation of microtubule attachments. Mutations in the NTR had defects in chromosome biorientation. To investigate how the NTR promotes accurate chromosome orientation and kinetochore-microtubule attachments, phosphomimetic and phosphodefective mutants were made

to study the effects on phosphorylation of SPC105R. MPS1-GFP localization was measured in SPC105R region specific deletion mutations to determine the interaction between SPC105R and MPS1. The deletion of several domains of SPC105R resulted in reduction of MPS1 localization, however no single domain has yet been determined to completely abolish MPS1 localization. Determining the function of SPC105R and its mechanisms will provide valuable insight into why nondisjunction occurs and how homologous chromosomes segregate properly.

716T **The role of the RNA polymerase I and III subunit Polr1D in ecdysone-mediated developmental transitions in *Drosophila melanogaster*** Bridget Walker, Ryan Palumbo, Bruce KnutsonSUNY Upstate Medical University

RNA Polymerases (Pols) I and III transcribe rRNAs, the building blocks of ribosomes. Assembly of both Pols begins with the formation of a heterodimer comprising POLR1D and POLR1C. Treacher Collins Syndrome (TCS) is craniofacial disorder caused by POLR1C and POLR1D mutations which can disrupt heterodimer formation and/or Pol complex integrity, and reduce rRNA expression and ribosome biogenesis. Consequently, this disturbance causes defects in neural crest cell proliferation and migration resulting in the malformation of craniofacial features. Previously, we established *Drosophila melanogaster* as a model system to study the role of POLR1D in development and in disease. A clinically-relevant TCS mutation in *Drosophila* Polr1D reduced rRNA levels and larval growth rate, and arrested larval development at the L2 stage. Arrested larval development is a phenotype observed in larvae mutant for genes in the ecdysone signaling pathway. Moreover, an RNAi screen in different tissues yielded additional phenotypes reminiscent of ecdysone pathway mutants. The steroid hormone ecdysone induces a transcriptional cascade of ecdysone response genes that drive developmental transitions and is functionally analogous to thyroid hormone in humans. Here, we hypothesized that Polr1D might play a specific role in the ecdysone signaling pathway. To test this, we utilized the UAS/GAL4 system to knock *Polr1D* down by RNAi specifically in the prothoracic gland (PG), the organ which produces ecdysone. We found that *Polr1D* RNAi knockdown in the PG yielded several phenotypes consistent with defective ecdysone signaling during the L3 stage, including large larval size, and a failure of L3 larvae to initiate wandering behavior and pupariation. Importantly, all of these phenotypes were rescued by feeding larvae 20-hydroxyecdysone (20E), the active metabolite form of ecdysone. Overall, these findings suggest that Polr1D plays a role in promoting the ecdysone pathway, and indicate a possible link between POLR1D and steroid hormone signaling in humans.

717F **The spindle assembly checkpoint limits brain size reduction in a fly model of human microcephaly** Constanza Mannino[1], Todd A Schoborg[21]Molecular Biology, University of Wyoming, [2]University of Wyoming

In the developing fly brain, neuroblasts undergo multiple rounds of asymmetric division during the neurogenic window of development in order to generate the neurons and glia of the nervous system. Cell number is the predominant scaling factor that determines overall brain size, thus the cellular mechanisms that influence the rate at which neuroblasts can divide may significantly impact brain growth and development. Mutations in the *abnormal spindle* gene disrupt mitotic spindle formation and proper division of neuroblasts and serves as a model for human primary microcephaly (MCPH), characterized by reduced brain size. However, whether *asp*-induced mitotic defects contributes to the MCPH phenotype remains poorly characterized. To better understand the role of mitotic defects in *asp* MCPH, we performed live-cell imaging of larval neuroblasts. We found a significant delay (~10x) in the metaphase to anaphase transition in *asp* mutants and an increase in the overall mitotic index, resulting from the activation of the spindle assembly checkpoint (SAC). To test whether SAC activation contributes to the *asp* MCPH phenotype, we generated *mad2/asp* double mutant animals. This restored the mitotic index to wildtype levels, but enhanced the MCPH phenotype as both larval and adult *mad2/asp* brains were significantly smaller than *asp* single mutants. We also observed a significant increase in the number of vacuole structures in the double mutants, resulting from hyperlocalized death of neurons and glia. Ongoing work is further probing the mechanisms of SAC activation in *asp* mutants, although our current data supports a model where the SAC has a 'protective' function in *asp* MCPH by limiting improper chromosome segregation and apoptosis, thus preventing a further decrease in overall brain size.

718F **Assessing cell type specific roles of *Abnormal spindle* in brain growth and development** Shalini Chakraborty, Todd Andrew SchoborgUniversity of Wyoming

Autosomal recessive primary microcephaly (MCPH) is a congenital condition which is characterized by reduction in brain size, intellectual disabilities, and life span. 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 this disorder remains unknown. To address this gap, we utilized the GAL4/UAS system to perform cell-type specific

genetic rescue assays to identify the cell type(s) that are responsible for the small brain phenotype. Expression of 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 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 (medulla, lobula, and lobula plate) 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. These results suggest that *asp* promotes proper brain growth through distinct neural stem cell populations in each adult brain region, and that this occurs in a non-cell autonomous fashion in the developing larval optic lobe. They also suggest the involvement of optic lobe paracrine signaling pathways whose identities are currently being investigated, as well as the mechanism by which Asp is able to regulate their signaling behavior.

719F **Whole genome approaches to understanding meiotic recombination** Carolyn Turcotte[1], Jeff Sekelsky[21]Genetics and Molecular Biology, University of North Carolina - Chapel Hill, [2]Biology, University of North Carolina - Chapel Hill

During meiosis, crossovers between homologous chromosomes ensure proper chromosome segregation and prevent chromosomal abnormalities in offspring. Crossovers are formed by repairing double-strand DNA breaks (DSBs) via homologous recombination. To ensure proper homolog segregation, the number and spatial arrangement of crossovers is tightly regulated in a phenomenon known as “crossover patterning.” Pathway choices within homologous recombination are traceable in products via heteroduplex DNA (hDNA), DNA in which the strands come from different parental chromosomes. The classic 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 classic model, ligated dHJs give rise to all crossovers by being cleaved in one of two patterns, generating two possible hDNA signatures. The model predicts that both patterns are equally likely, yet only one of the hDNA signatures has been observed. This hDNA signature bias demands revision of the meiotic recombination model. 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. Illuminating the interplay between meiotic recombination and crossover regulation is essential to understanding the mechanisms behind chromosome segregation and propagation of sexually reproducing organisms.

720F **Synergistic tumorigenesis by Src and Yki via inhibition of *Socs36E* and *hid*** Keigo Ogawa, Masato Enomoto, Tatsushi IgakiKyoto University

The expression and activity of the oncogene Src are frequently elevated in many types of human cancers. However, the mechanism by which Src contributes to tumor progression *in vivo* is still elusive. We have previously found in *Drosophila* imaginal discs that clones of cells overexpressing Src64B (a c-Src homolog) cause cell death when surrounded by wild-type cells and were eventually eliminated from the tissue. Here, we found that simultaneous mutation of the tumor suppressor gene *hippo* (*hpo*) not only blocks the elimination but causes synergistic tumor growth of Src-activated cells. We found that tumor overgrowth of Src/hpo requires microRNA *bantam* that is induced by Yorkie (Yki), a downstream of effector of the Hippo pathway. Furthermore, we identified *Socs36E*, a negative regulator of the JAK/STAT pathway, and the cell death gene *hid* as target genes of *bantam* as minimal requirements for the synergistic tumor growth caused by Src and Yki activation. The mechanism underlying the synergistic tumor growth will be discussed.

721F **Two different two-hit EMS screens on chromosome 2R and 2L generate growth and developmental mutations that are characterized and mapped by undergraduate researchers in the Fly-CURE** Joyce Stamm[1], Jacob Kagey[2], Kelsey Breneman[1], Audrey Chambers[1], Emily Daniel[2], Brooke Garland[3], Ayyat Khan[2], Annie Kim[1], Stephanie Korte[21]Biology, University of Evansville, [2]University of Detroit Mercy, [3]ReBUILDetroit, University of Detroit Mercy

Fly-CURE is an NSF-funded project in which undergraduate students participate in the phenotypic characterization and mapping of novel *Drosophila* mutants within a classroom research experience. To support this project, we have generated EMS mutants for two Flp/FRT genetic screens, one each on chromosomes 2R and 2L, to identify genes that regulate cell growth and division during development. Both screens were carried out in a background of blocked cell death by transposon-insertion mutations in *Dark (Drosophila apoptosis-related factor),* and *AIF (apoptosis-inducing factor)* for the 2R and 2L screens respectively. Despite both Dark and AIF having previously defined roles in apoptosis,

the mosaic phenotype that each creates is dramatically different. For *Dark*, there is a small but consistent increase in the amount of mutant tissue, allowing us to screen for secondary growth mutations that are conditional upon a block in apoptosis. In contrast, the *AIF* mosaic eye results in a strong reduction in the amount of mutant tissue, allowing us to screen for genetic enhancers and suppressors of this phenotype. Both transposon generated mutants (*Dark* and *AIF*) carry a *white*$^{+}$ marker, *eyeless-FLP* therefore generates red double-mutant clones. To date, students in Fly-CURE have successfully mapped and characterized 15 mutations on 2R, and mapped them to a variety of genes, including *Shn, Hpo, Cid, Egfr*, and *Ptc*. Characterization of the first few 2L mutants is ongoing.

722F **Exploring protein interactions in the ERK/MAPK signaling pathway with *in vivo* bimolecular fluorescence complementation (BiFC)** Maddison Lessard, Claire S Jackan, Alexey VeraksaDepartment of Biology, University of Massachusetts Boston

ERK/MAPK signaling controls cell proliferation and differentiation by regulating the activity of various transcription factors. A key target of ERK regulation is Capicua (Cic), a transcriptional repressor protein that controls tissue patterning during embryonic development in flies. ERK phosphorylates Cic to relieve repression and thereby promotes cell division and differentiation. We are investigating the interaction between the ERK and Cic proteins with bimolecular fluorescence complementation (BiFC) assays. BiFC relies on the use of a split fluorescence protein such as Venus that only emits light if the two tagged proteins interact. We have found that ERK and Cic interact primarily in the nucleus in various larval tissues, and have validated a previously identified ERK binding site on Cic *in vivo*. ERK and Cic form subnuclear puncta in our BiFC assay, and we are currently investigating their properties and possible functions. We are also exploring how manipulating ERK pathway activation affects ERK-Cic interaction in fly larvae and embryos. This work contributes to the *in vivo* characterization of key protein interactions that control proliferation and patterning during development.

723F **The effects of induced endocycling cells on tissue growth and function** Hunter Herriage, Yi-Ting Huang, Padma Rangarajan, Brian R CalviBiology, Indiana University

Developmental endocycling cells (devECs) support tissue growth in many organisms. These devECs have oscillating growth (G) and synthesis (S) phases without an intervening mitotic (M) phase, which results in large polyploid cells. In contrast to programmed devECs, conditional signals can also induce mitotic cycling cells into an endocycle, for example during aging, wound healing, or cancer. While these induced endocycling cells (iECs) can be beneficial in tissue repair and regeneration, they can also contribute to disease. We previously showed that endocycling cells resist apoptosis, and, unlike most devECs, iECs can return to an error-prone mitosis (RTM) that generates aneuploid daughter cells. Evidence now suggests that these iEC properties contribute to cancer therapy resistance and tumor regrowth. Much remains unknown, however, about how iECs impact tissue growth and function in development and disease.

We have been evaluating how iECs can affect the growth and function of different fly tissues. We examined the ovarian follicular epithelium that surrounds the germline of growing egg chambers. Follicle cells initially proliferate mitotically, but then switch to developmental endocycles in stage 6/7 of oogenesis and subsequently increase in cell size and ploidy. Both mitotic cycling and endocycling follicle cells form a normal epithelium in which cells have a uniform shape and polarity. In contrast to these devECs, we find that induction of a precocious endocycle before stage 6 perturbs the architecture of the follicular epithelium. It is known that defects in epithelial integrity contribute to oncogenesis. There was no evidence, however, for tissue overgrowth when recovering iECs returned to mitosis. To determine how iECs affect the function and growth of other tissues, we induced larval brain stem cells (neuroblasts) into the endocycle. It has been shown that neuroblasts can undergo hyperplasia upon loss of cell polarity or division integrity. We find that inhibition of mitosis switches neuroblasts into the endocycle, which results in highly polyploid neuroblast iECs. These iECs have defects in stem cell polarity. We are currently evaluating the impact of induced endocycles on stem cell identity and function, and whether these iECs can contribute to tissue overgrowth upon return to mitosis. We will discuss how the observed effects of iECs on cell and tissue architecture suggest a new model for how unscheduled endocycles can contribute to tumorigenesis.

724F **The effect of induced endocycling cells on tissue growth and homeostasis** Yi-Ting Huang, Lauren L. Hesting, Hunter C. Herriage, Brian R. CalviIndiana University Bloomington

The endocycle is an alternative cell cycle during which cells undergo repeated rounds of S and G phases without division, which results in large, polyploid cells. Endocycles contribute to the normal growth of specific tissues through an increase in cell size instead of cell number (hypertrophy). In addition to these programmed developmental endocycling cells (devECs), cells can switch from mitotic cycles to endocycles in response to stress and other conditional signals, which

we call induced endocycling cells (iECs). We have previously found that both devECs and iECs repress the apoptotic response to genotoxic stress. We also found that, unlike most devECs, iECs can return to an error-prone mitotic cycle that generates aneuploid daughter cells. It is now clear that these iEC properties of cell survival and genome instability are shared with endocycling cancer cells and contribute to cancer therapy resistance and tumor regrowth. Thus, understanding how iECs affect tissue growth during development is an important question. To address this, we have been evaluating the impact of iECs on tissue growth in the *Drosophila* wing disc. We induced cells into the endocycle through genetic mosaic inhibition of mitosis. The resulting iEC clones subsequently grow in cell size instead of cell number. iEC clones initially accumulate tissue mass at a rate that is proportional to mitotically-dividing control clones, but then their cell growth slows and their increase in clone size lags behind controls. We find that iECs accumulate different cell stress markers and, although they repress apoptosis, they ultimately respond to this stress by engaging a senescent-like arrest. We also found that iEC growth differed among regions of the wing disc, suggesting that developmental context influences their growth rate and final cell size. We are currently evaluating how regional signals influence the growth of iECs, and how, in turn, iECs influence growth of neighboring diploid cells. Altogether, our results suggest that an increase in cell size of unscheduled, induced endocycling cells can contribute to tissue growth through hypertrophy, but ultimately these cells arrest in response to stress and do not fully compensate for tissue growth that normally occurs through an increase in cell number. Our results also have broader impact for understanding how unscheduled iECs contribute to human developmental anomalies and tumor growth.

725F **Mechanism of natural variation in double strand break repair** Shahrzad Hajiarbabi, Erin KelleherBiology and Biochemistry, University of Houston

DNA repair is critical to maintain genome sequence fidelity in the face of endogenous and environmental mutagens, yet individuals often differ in their capacity to repair different forms of DNA damage. We previously mapped natural genetic variation in sensitivity to double stranded breaks (DSBs) to a large locus near the 2nd chromosome centromere. Mutagen sensitive alleles show decreased viability following X-ray radiation exposure, and increased sterility following germline mobilization of *P*-element DNA transposons, both of which suggest differences in DSB repair. However, the repair pathway whose reduced function explains these mutagen-sensitive phenotypes remains unknown. To uncover the mechanism of mutagen sensitivity, I am comparing mutagen sensitive and tolerant alleles with respect to 1) repair products of double stranded breaks and 2) sensitivity to a broad range of chemical mutagen classes.

DSBs in *Drosophila melanogaster* are repaired through both homologous (HR) and non-homologous end joining (NHEJ) pathways. To identify which of these two types of pathways exhibits reduced function as a consequence of mutagen-sensitive alleles, I am employing a gap repair assay, in which repair products resulting from HR and NHEJ yield diagnostic eye color phenotypes. I will further examine whether these two alleles differ in sensitivity to other forms of DNA damage in addition to DSBs, by treating them with a range of chemical mutagens that cause different types of lesions in DNA. While mutant alleles of some repair factors show broad sensitivity to chemical mutagens, others are only sensitive to a particular mutagen class, indicative of inability to repair a particular lesion type. Together, my experiments will point to the host-factors whose differential activity determines sensitivity to DNA damage. These “mutator alleles” play important roles in mutation rate evolution as well as organismal responses to genotoxic stress.

726F **The coactivator Taiman modulates cell competition via glypican-dependent diffusion and availability of the Wg morphogen** Colby K Schweibenz, Ken H MobergCell 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 the Ecdysone receptor and the Hippo pathway effector Yorkie, are able to 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 wing and eye disc to test the reciprocal hypothesis, that cells with reduced Tai expression (Tai^{low}) are competitive ‘losers,’ and we use a genetic screen to identify candidate mechanisms required for elimination of these cells by wildtype neighbors. This screen recovered ‘hits’ in pro-apoptotic genes (*head involution defective*, *reaper,* and *grim*) as dominant suppressors of the Tai^{low} loser phenotype, confirming a dependance on apoptosis. Notably this screen also recovered the two *Drosophila* Adenomatous polyposis coli (APC) tumor suppressor homologs, Apc1 and Apc2, which are conserved elements of the Wg pathway and inhibit field cancerization in the fly midgut. Apc1/Apc2 loss rescues elimination of Tai^{low} cells in both eye and wing epithelia, arguing that Tai loss may reduce Wg signaling. Consistent with this model, we find that Tai is required for expression of

a Wg gene target (*naked cuticle*) in larval wing cells, and that Tai promotes expression of genes encoding the glypicans (GPI-anchored HSPGs) Dally and Dally-like protein (Dlp), which respectively enable short or long-range Wg signaling. Thus, we postulate Tai^{low} cells become losers due to reduced expression of Dally/Dlp, which in turn leads to reduced capture of extracellular Wg by Tai^{low} cells relative to adjacent wildtype cells. Ongoing experiments seek to establish mechanistic links between Tai and Dally/Dally-like expression, with the goal of defining how Tai modulates winner/loser status by remodeling the extracellular matrix.

727F **Coordination of DNA synthesis during homologous recombination repair of large gaps** Meaghan Dineen, Bridget Walker, Nicole Gubitosi, Daniel KaneLe Moyne College

DNA damage can lead to mutations and even genetic diseases and disorders if not repaired properly. DNA double strand breaks (DSBs) are especially problematic lesions as flanking sequence to the break can be mutated or lost. More accurate repair by the homologous recombination (HR) pathway involves synthesis of new DNA to recover any lost information. 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 novel 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 the former being utilized in longer synthesis tract lengths than the latter. In our current research, we aim to determine what factors upstream of the DNA polymerases may be coordinating the selection of these different polymerase types for utilization at sites of DSBs. We are examining three Drosophila mutants: one polymerase mutant with a deleted C-terminal domain (*rev1ΔCTD*) that does not impact its catalytic function but does impact its ability to interact with other polymerases, and two mutants eliminating function of various polymerase clamps (*PCNA2* and *hus1*). Preliminary data shows that the *rev1ΔCTD* phenotype matches the previously studied *rev1* further supporting that Rev1 may be used in recruiting and coordinating translesion polymerase activity. Early trial attempts to obtain homozygous *PCNA2* mutants with a generated DSB by *P{w^a}* excision were unsuccessful and will be investigated further to see if this suggests a possible synthetic lethality. Lastly, and of particular interest, preliminary analysis of *hus1* shows remarkable similarity to the previous *pol32* phenotype suggesting an active role in aiding polymerase delta synthesis during HR, beyond just a role of checkpoint control seen in this assay with regulators like *mei-41* (ATR). We will be increasing the sample sizes in our analyses to determine if these proteins do indeed play a role in DNA polymerase choice during homologous recombination in DSB repair, facilitating synthesis of longer repair tracts with replicative polymerases via Hus1 or shorter repair tracts with translesion polymerases via Rev1.

728F **The binding sites of E2F transcription factor in *Drosophila* metabolic genes are functionally distinct** Maria Paula Zappia[1], Yong-Jae Kwon[1], Anton Westacott[1], Isabel Liseth[1], Hyun Min Lee[1], Abul B.M.M.K. Islam[2], Jiyeon Kim[1], Maxim Frolov[11]Biochemistry and Molecular Genetics, University of Illinois at Chicago, [2]Department of Genetic Engineering and Biotechnology, University of Dhaka

The canonical role of the transcription factor E2F is to control the expression of cell cycle genes. E2F binds to its motif sequence in the promoter and regulatory regions of thousands of genes, including metabolic genes. Many of these genes contain E2F consensus sequences and their expression is altered upon E2F inactivation. These findings raise an important question. How much do E2F motif sequences contribute to the binding of E2F and the regulation of gene expression in vivo? To explore the role of E2F in controlling the expression of metabolic genes, we used the CRISPR/Cas9 technology to introduce point mutations in the E2F motif sites upstream of five endogenous metabolic genes in*Drosophila*. We found that the impact of mutating the E2F sites had a wide range of effect in the recruitment of E2F and the expression of the target genes. Remarkably, the glycolytic gene, *Phosphoglycerate kinase* (*Pgk),* was among the most affected gene. The loss of E2F regulation on *Pgk* gene led to metabolic alterations, including a decrease in glycolytic flux, in levels of TCA cycle intermediates and in content of ATP. Strikingly, chromatin accessibility was significantly reduced at multiple genomic regions in $Pgk^{\Delta E2F}$ mutants. These regions contained hundreds of genes, including metabolic genes that were downregulated in $Pgk^{\Delta E2F}$ mutants. Moreover, $Pgk^{\Delta E2F}$ animals had shortened life span, low glycogen content, and exhibited defects in the high-energy consuming organs, ovaries and muscles. In ovaries, the egg chambers degenerated at the onset of vitellogenesis and produced poor quality eggs, whereas in muscles, the mitochondrial morphology was abnormal leading to dysfunctional muscles. Collectively, our results illustrate how the pleiotropic effects on metabolism, gene expression and development in the $Pgk^{\Delta E2F}$ animals underscore the importance of E2F regulation on a single E2F target, *Pgk*.

729F **Nuclear reassembly defects after mitosis trigger an apoptotic safeguard mechanism in *Drosophila*** Jingjing Li[1,2], Laia Jordana[2,3], Haytham Mehsen[3], Virginie Emond-fraser[2,3], Xinyue Wang[3], Vincent Archambault[2,31]Institute for

research in immunology and cancer, [2]Département de biochimie et médecine moléculaire, Université de Montréal, [3]University of Montreal, Institute for research in immunology and cancer

In animals, mitosis requires the breakdown of the nuclear envelope and the sorting of individualized, condensed chromosomes. During mitotic exit, nascent nuclei reassemble a nuclear envelope around a single mass of interconnecting chromosomes. Defects in this process can result in aberrant nuclei with abnormal structure and function. While the molecular mechanisms of nuclear envelope reformation are emerging, the cellular and physiological consequences of defects in this process are poorly understood. We took advantages of perturbations in the Ankle2-BAF mechanism to investigate this question. BAF plays a central role in nuclear reassembly. It is a DNA-binding dimeric protein that interconnects chromosomes in telophase to promote the assembly of a single nucleus. BAF also binds lamins and transmembrane proteins of the nuclear envelope. During mitotic entry, BAF phosphorylation disrupts its interactions with chromatin. Ankle2 is required for PP2A-dependent BAF recruitment on reassembling nuclei in *C. elegans* and in human cells. We confirmed that this function of Ankle2 is conserved in *Drosophila*. We found that partial depletion of Ankle2, BAF or Lamin in *Drosophila* imaginal wing discs results in nuclear reassembly defects that trigger apoptosis and cause wing development defects. Using a genetic approach, we searched for signaling pathways that become critical for wing development in this context. Among several functional interactions identified, blocking apoptosis had the most profound effect, strongly enhancing wing development defects. Our results suggest that an apoptotic response to sporadic nuclear reassembly defects plays a crucial role in safeguarding tissue development.

730S **Quantifying Histone H3's role as a Competitive Inhibitor of Chk1 in the Early Embryo** Kiera E Schwarz, Yuki Shindo, Amanda A AmodeoBiology, Dartmouth

In order to properly function, cells must follow a variety of cues to properly time their divisions. In the first hours of development, Drosophila embryos undergo 13 cycles of rapid, syncytial, nuclear divisions, in which many cellular checkpoints are bypassed in favor of faster development. The DNA damage checkpoint kinase Chk1 is inactive until the Mid-Blastula Transition (MBT), in which the embryo cellularizes and begins slower cellular divisions and integrates gap phases with multiple cellular checkpoints. Once activated, Chk1 phosphorylates downstream target Cdc25 impeding cell cycle progression. The onset of cell cycle slowing at the MBT is dependent on the nuclear to cytoplasm (N/C) ratio, which in turn times the activation of Chk1 based on growing syncytium size. Our lab has recently shown that histone H3-tail acts as a competitive inhibitor of Chk1, and when overexpressed creates faster cell cycles and delays the MBT. H3 is rapidly incorporated into nucleosomes as the number of nuclei increases, inactivating Chk1 until the correct N/C ratio is met. In this work we will measure the in vivo concentrations of essential cell cycle regulators in Drosophila Cdc25 isoforms (String and Twine), Chk1 (Grps), and H3-tail, as well as their respective in-vitro binding affinities to Chk1 through surface plasmon resonance. We are also currently studying the impact of phosphomimetic and non-phosphorylatable mutations of H3-tail in vivo and in vitro. These quantifications will help define the kinetics of H3-tail inhibition of Chk1, and allow us to better understand the interplay of histones and the canonical Chk1-Cdc25 pathway in cell size sensing in the early embryo.

731S **Cooperative Regulation of Growth by Defective Proventriculus and Yorkie in the *Drosophila* eye** Rohith BN[1], Neha Gogia[1], Arushi Rai[1], Madhuri Kango-Singh[1,2,3], Amit Singh[1,2,31]Department of Biology, University of Dayton, [2]Premedical Program, University of Dayton, [3]Integrative Science and Engineering Center (ISE), 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. Axial patterning precedes differentiation in the *Drosophila* eye which begins from a ventral equivalent state. The dorsal fate is established by onset of expression of the GATA-family transcription factor Pannier (Pnr), and other dorsal-specific genes like Iroquois (Iro-C) family proteins. 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 repressor that binds to the K50 site. 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*? *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. We tested if these pathways act independently to control eye patterning and growth or act via shared targets and regulatory interactions. In this context we investigated the effect of overexpressing Yorkie (the effector of the

Hippo pathway) and Dve during larval development specifically in the dorsoventral domains of the eye imaginal discs. Overexpression of *yki* extends the *dve* domain in the eye disc as a result of which the disc is enlarged, and suppresses eye differentiation, thereby suggesting that these two distinct genes may regulate a common downstream target to control the disc growth and differentiation. We have tested *wingless* (*wg*) a known and conserved Hippo downstream target for *dve* and *yki* mediated effects using reporter assays, and qRT-PCR- based approaches; and our results will be discussed.

732S ***The role of Jagunal in regulating stemness of neuroblast during embryonic-to-larval transition of Drosophila*** Judy Especial Abuel[1], Aracely Martinez Peraza[21]Biology, San Francisco State University, [2]San Francisco State University

The process of cell division raises many questions related to how cells become specialized as well as what mechanisms are involved in cell fate differentiation. Recent study shows that asymmetric cell division (ACD) of the ER during mitosis depends on a highly conserved ER membrane protein Jagunal (Jagn). In addition, cell polarity is necessary for the correct partitioning of cell fate determinants apically and basally. However, the mechanism or signaling pathway by which these cell fate determinants are positioned is poorly understood. Here, we propose that Jagn plays a role in organizing basal cell fate determinants towards cellular differentiation. This is largely based on preliminary data that Jagn-deficient larval brains display a shift towards stemness with an over proliferation of neuroblast. In order to understand this over proliferation of neuroblast, we focused on the maintenance of the quiescent state of neuroblast during the embryonic-to-larval transition. In order to investigate this, we performed immunostaining with Deadpan (Dpn, neuroblast marker) and EdU incorporation (quiescence marker) using neuroblast drivers *worniu (wor)*-Gal4, Inscuteable (Insc)-Gal4, and Delta-Gal4 in combination with UAS-Jagn RNAi in stage 15 embryos. Embryos were fixed, stained, and processed for imaging using a confocal microscope. Our results show an increase in proliferation of neuroblast in Jagn-deficient embryos, suggesting a lack of quiescence during the transition to larval development. Future directions will focus on the role of Jagn in the regulation of the embryonic quiescence state and the correct partitioning of cell fate determinants involved in cellular differentiation.

733S **Understanding the role of *matrimony* in suppressing the drive of the B chromosomes** Kaylah B Samuelson[1], Ryan Gado[1], Allison Gardner[1], Stacey L Hanlon[1,21]Molecular and Cell Biology, University of Connecticut, [2]Institute of Systems Genomics, University of Connecticut

The genome is under relentless assault by selfish genetic elements that bias their transmission into the next generation at the expense of their host. A classic example of selfish genetic elements are B chromosomes, which are supernumerary, nonessential chromosomes that have been shown to promote their own segregation to ensure their inheritance despite imposing a fitness cost. Recently B chromosomes were discovered in a single laboratory stock of *D. melanogaster* that carries a null mutation in *matrimony* (*mtrm*^{126}) held over a third chromosome balancer (*TM3, Sb Ser*). This combination allows the B chromosomes to be transmitted through female meiosis at a higher-than-expected frequency, a phenomenon referred to as meiotic drive. While it is clear that having only one functional copy of *mtrm* is necessary but not sufficient for this drive, it is unknown how a reduction in Mtrm leads to drive of the B chromosomes. Mtrm promotes centromeric cohesion of achiasmate (non-crossover) chromosomes during female meiosis and ensures their proper segregation during the meiotic divisions by regulating Polo kinase (Polo) in a 1:1 genetic ratio. Since the genetic levels of *mtrm* and *polo* are crucial for proper achiasmate chromosome segregation, we genetically altered the ratio of *mtrm* and *polo* by combining a null allele of *polo* (*polo*$^{16\text{-}1}$) with *mtrm*^{126} (*mtrm*^{126} *polo*$^{16\text{-}1}$/*TM3*) to determine if restoring the *mtrm:polo* ratio back to 1:1 rescues drive suppression of the B chromosomes. We will show the comparison of B chromosome transmission frequencies in the recombinant background (*mtrm*^{126} *polo*$^{16\text{-}1}$/*TM3*), a drive-permissive background (*mtrm*^{126}/*TM3*), and a *polo*$^{16\text{-}1}$/*TM3* background. We also tested whether the interaction between Mtrm and Polo is necessary to suppress the drive of the B chromosomes, since their interaction is necessary to promote the proper segregation of achiasmate chromosomes. We will show the comparison of B chromosome transmission frequencies in a drive-competent (*mtrm*^{126}/*TM3*) genetic background both in the presence and absence of Mtrm transgenes that affect Mtrm's ability to bind Polo (e.g., *mtrm*^{T40A}). Our investigation will illuminate how the interaction between Mtrm and Polo influences the meiotic drive of the B chromosomes. Overall, we are working towards uncovering the mechanisms behind how the host genome can protect itself against the spread of selfish genetic elements.

734S **B chromosome dynamics during female meiosis in *D. melanogaster*** Mengjia Lin[1], Stacey Hanlon[1,21]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

B chromosomes are extra, non-essential chromosomes that are found in hundreds of species including fungi, animals, and plants. B chromosomes are typically heterochromatic and carry satellite repeats and transposons, and are often not required for normal growth and development. Recently, B chromosomes were discovered in a single laboratory stock of *D. melanogaster*, which carries a null mutation in *matrimony* (*mtrm*^{126}) held over a third chromosome balancer (*TM3, Sb Ser*). In this stock, B chromosomes are preferentially passed to the next generation with a transmission frequency of 64%, thereby defying Mendelian inheritance and exhibiting a behavior known as meiotic drive. When the B chromosomes are put into a wild-type background, the transmission frequency was closer to Mendelian expectations (52%), indicating that the stock background—and not the B chromosomes—is contributing to the meiotic drive of the B chromosomes. Consistent with this observation the B chromosomes display an abnormal arrangement during the metaphase I arrest in the mutant background but not in a wild-type background, but how this abnormal arrangement results in the biased transmission of the B chromosomes remains unknown. To uncover how the B chromosomes are segregating during the meiotic divisions, we performed fluorescent *in situ* hybridization and immunofluorescence to visualize the chromosomes and the meiotic spindle in newly activated eggs that are still undergoing the meiotic divisions. Our goal is to observe where the B chromosomes are at each stage of meiosis, thereby creating a timeline of their dynamics and enabling us to form hypotheses as to how the mutant background is influencing their aberrant segregation. Understanding how these B chromosomes segregate with bias during female meiosis will provide critical insight into how non-essential, newly formed chromosomes may be able to remain in a population despite not being able to promote their own meiotic drive.

735S **Exploring the relationship between the B chromosomes and Chromosome 4 segregation** Shell Chen[1], Ayushi A Patel[1], Stacey L Hanlon[1,21]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

B chromosomes are supernumerary genetic elements that are carried by hundreds of different species. Recently, B chromosomes were discovered in a single laboratory stock of *Drosophila melanogaster* and are held at a high copy number (10-12 B chromosomes). The B chromosomes do not carry genic regions but do carry the *AAGAT* satellite repeat, indicating that they likely formed after a breakage event on Chromosome 4. The presence of B chromosomes causes a significant increase in the frequency of Chromosome 4 nondisjunction during female meiosis, although it is unclear if the abundance of B chromosomes or their presence *per se* is promoting the improper segregation of Chromosome 4. Conversely, whether the B chromosomes segregate in a Mendelian fashion when Chromosome 4 segregation is abnormal is also unknown. To probe the relationship between the B chromosomes and Chromosome 4 segregation, wild-type females with B chromosomes were crossed to males that carry a compound Chromosome 4, enabling us to distinguish between progeny that received anormal (one) or abnormal (zero or two) number of Chromosome 4s from the female parent. The ovary tips from each parental female were squashed and her B chromosome copy number was determined by counting the number of B chromosomes present in pre-meiotic mitotic metaphases. The B chromosome copy number was also determined for her progeny that received zero, one, or two copies of Chromosome 4. Together, we will present data on how the number of B chromosomes present during female meiosis influences the segregation of Chromosome 4. A linear correlation would indicate that the number of B chromosomes affects how severe Chromosome 4 segregation is affected, whereas a non-linear relationship would suggest that the presence of the B chromosomes, regardless of their copy number, disrupts how Chromosome 4 segregates. We will also present our findings on how the B chromosomes segregate when Chromosome 4 segregates abnormally, which will provide further insight into the relationship between these two chromosomes. Combined, the results of this work will allow us to begin unraveling how the B chromosomes are able to disrupt the segregation of the essential chromosomes during female meiosis.

736S **Determining how the *TM3, Sb Ser* balancer chromosome contributes to the meiotic drive of the B chromosomes in *D. melanogaster*** Ryan M Gado[1], Stacey L Hanlon[1,21]Molecular and Cell Biology, University of Connecticut, [2]Institute for Systems Genomics, University of Connecticut

B chromosomes are extra, non-essential chromosomes that often behave as selfish genetic elements and employ complex mechanisms to ensure they are transmitted from parent to progeny at a high frequency. In *D. melanogaster*, B chromosomes were recently found in a laboratory stock that carries a null allele of *matrimony* (*mtrm*^{126}) over the *TM3, Sb Ser* (*TM3*) balancer. In this original stock, the B chromosomes violate Mendel's Law of Segregation and are preferentially passed to progeny during female meiosis. This phenomenon, known as meiotic drive, is assessed by evaluating the transmission of the B chromosomes from parent to progeny. It has been shown that the presence of both the *mtrm*^{126} allele and the *TM3* balancer are permissive for the robust drive of the B chromosomes; however, it is unknown how the *TM3* balancer contributes to meiotic drive. Additionally, screening through a pool of candidate drive enhancers using the traditional method of determining B chromosome copy number would take an immense amount of time since the assay

requires a lengthy process of ovary dissections, fluorescence *in situ* hybridization, and imaging to cytologically count the B chromosomes. Therefore, to rapidly test candidate genes and determine which are complicit in *TM3's* enhancement of meiotic drive, we are establishing a molecular assay that assesses B chromosome copy number using digital droplet PCR (ddPCR), which provides the resolution we require to distinguish between high copy numbers of B chromosomes. Though there are currently a handful of unique sequences we can target on the B chromosome, we are also aiming to tag the B chromosomes with a P element that is genetically marked and would provide another series of unique ddPCR target sites. We are also exploring if the readout of Chromosome 4 segregation in the absence of B chromosomes mimics the biased transmission we observe with the B chromosomes, which may allow us to convert Chromosome 4 nondisjunction into a transmission frequency and use it as a proxy for B chromosome segregation in our screen of candidate drive enhancers. Ultimately, this work will provide the starting point for uncovering how the *TM3, Sb Ser* balancer chromosome is enhancing the meiotic drive of the B chromosomes.

737S **Investigating Drosophila melanogaster Glial Cell Deveopment and Oncogenesis using UAS/GAL4** Irene Hsu, Renee ReadEmory University

Glioblastoma is the most aggressive and common form of primary brain cancer. Unfortunately, current treatments of surgical resection, radiation therapy, and chemotherapy still yield a poor prognosis of 15 months median survival. By gaining a better understanding of normal glial cell subtypes underlying glioblastoma development, we may be able to develop new strategies to target subtypes of tumorigenic glia to optimize new treatments. *Drosophila melanogaster* has a highly conserved nervous system, with easily identified glial cell types that can be used to model mammalian glia as well as glioblastoma. In this project, we identified subtypes of glial cells in the larval *Drosophila* brain with the Gal4/UAS-GFP system. By using different Gal4 enhancer traps, we expressed UAS-GFP in a glial cell type specific manner and visualized morphology and development of glial cell subtypes using confocal microscopy. To perform quantitative analysis on tumor that arise from different subtypes of glial cells, we will use Gal4/UAS to express glioblastoma-causing oncogenes. We expect to see GFP expressed differentially across different subtypes of wild-type glia, and to see neoplastic changes in these cells when they overexpress glioblastoma-causing oncogenes. We suspect that cortex glia and astrocytes may be much more prone to neoplastic transformation than other glia, such as perineural or subperineural glia. We will also attempt to perform drug treatment on tumors derived from different subtypes of glioblastoma and qualitatively analyze changes in their growth and morphology. The results can further guide us in exploring aspects of mammalian tumor development and possible treatments for glioblastoma specific to subtypes of glia.

738S **Mapping of the B.3.4, M.3.2, and G.3.2 mutations in *Drosophila melanogaster*** Lauren Heininger[1], Adriana Andrus[1], Alex Caudill[1], Lauren James[1], Regan Landis[1], Emmily Moses[1], Reese Saho[1], Emma Widmer[1], Jacob Kagey[2], Jamie Siders[31]School of Science, Technology, and Mathematics, Ohio Northern University, [2]Biology, University of Detroit Mercy, [3]Ohio Northern University

The Fly-CURE is a Course-based Undergraduate Research Experience (CURE) that employs a Flp/FRT genetic screen in *Drosophila melanogaster* to identify homozygous lethal mutations on chromosome 2R contributing to cell growth and development. The current work describes the phenotypic characterization and genetic mapping of three of these EMS-induced mutations: B.3.4, M.3.2, and G.3.2. ;FRT42D,Dark82,mw+,B.3.4 /CyO, ;FRT42D,Dark82, mw+,M.3.2/CyO, and ;FRT42D,Dark82,mw+,G.3.2/CyO heterozygous stocks were crossed to w;FRT,Dark82;Ey>Flp to generate homozygous mutant clones (-/-;red) in the eye and each mutant resulted in distinct cell growth phenotypes. B.3.4 resulted in an increase of red>white pigment ratio, M.3.2 mutants exhibited cuticle overgrowth in conjunction with a red>white pigmentation, and G.3.2 mutants displayed a unique white>red phenotype. Complementation mapping data for each mutant established that B.3.4 maps to chromosomal location 2R:17739710..17767088 and G.3.2 maps to 2R:17394025..17462625. Deficiency crosses combined with crosses to single gene alleles demonstrated that M.3.2 maps to the *tout-velu* (*ttv*) gene locus. Future work will consist of single gene crosses to determine the mutant gene in B.3.4 and G.3.2 and genetic sequencing to confirm the identity of all three mutations.

739S **The histone chaperone NASP maintains H3-H4 reservoirs in the early Drosophila embryo** Reyhaneh Tirgar, Jonathan P Davies, Lars Plate, Jared NordmanVanderbilt University

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 but the chaperone necessary to stabilize soluble H3-H4 pools in the

Drosophila embryo has yet to be identified. Here, we show that CG8223, the Drosophila ortholog of NASP, is a H3-H4-specific chaperone in the early embryo. NASP specifically binds to H3-H4 in the early embryo. We demonstrate that, while a *NASP* null mutant is viable in Drosophila, *NASP* is a maternal effect gene. Embryos laid by NASP mutant mothers have a reduce rate of hatching and show defects in early embryogenesis. Critically, soluble H3-H4 pools are degraded in embryos laid by NASP mutant mothers. Our work identifies NASP as the critical H3-H4 histone chaperone in the Drosophila embryo.

740S **Defining the functions of p53 isoforms and p53 nuclear bodies** Padma Rangarajan[1], Brian R Calvi[21]Biology, Indiana University, [2]biology, Indiana University

The long list of p53 cellular functions continues to grow. We have previously shown that different *Drosophila* p53 isoforms have overlapping and distinct functions in apoptosis and autophagy. The physiological functions of the longest p53B isoform, however, had largely remained a mystery. To address this, we created fly strains with GFP-p53A and mCherry-p53B isoform fusions and isoform-specific p53 mutants using CRISPR / Cas9. We found that p53B expression is germline specific, and, with p53A, is required for repair of meiotic DNA breaks and oocyte quality control, with relevance to the functions of mammalian p63 and p53 in oogenesis. The analysis of the fluorescently-tagged p53 isoforms indicated that they form one prominent and several minor nuclear bodies, and that the abundance of p53 in these bodies changes during meiotic break repair. These results are reminiscent of the dynamic localization of human p53 to nuclear bodies, whose significance is still being defined. Our continued analysis indicates that the most prominent p53 nuclear body may associate with a chromosomal locus. Live and fixed cell imaging indicate that the p53 bodies are dynamic but continue to associate with condensed chromosomes in mitosis. These results lead to a working model wherein the functions of *Drosophila* p53 isoforms are mediated by their localization to phase separated nuclear bodies.

741V **Remote production of TNF-α/Eiger contributes to Myc super-competition in developing wing discs** Aditi Sharma Singh, Albana Kodra, Laura A. JohnstonGenetics and Development, Columbia University Medical Center

Cell competition is a surveillance mechanism that promotes tissue/organ fitness by allowing healthy cells to contribute to organ development at the expense of cells that are relatively less fit. Competitive interactions can benefit the tissue by eliminating viable, but less healthy cells, but can also be detrimental, as when oncogenic “super-competitor” cells take over an otherwise healthy tissue. We have developed a model of super-competition using *Drosophila* wing imaginal discs, wherein cells over-expressing the proto-oncogene Myc (“winners”) compete for space with wild-type (WT) cells (“losers”) during the rapid growth phase of larval development. The death and elimination of WT loser cells from the wing disc is mediated by cell-cell interactions that activate a cell competition signaling module (CCSM) in WT cells, leading to their death. We find that Eiger (Egr), the sole TNF in *Drosophila*, and the TNFR Grindelwald (Grnd) also conribute to elimination of the WT loser cells, independently of canonical JNK effectors. Moreover, although Grnd/TNFR is expressed in all wing disc cells, studies *in-vivo* with Egr reporters and GFP fusion proteins reveal no evidence of Egr expression in either the WT loser or Myc-expressing winner cells. Selective tissue-specific depletion of Egr/TNF reveals that production of Egr by the CNS and fat body, but not by hemocytes, is sufficient to activate loser cell death in wing discs. We will present these and other data from experiments that investigate how remotely produced Egr/TNF signals via Grnd/TNFR in wing discs, and how its activity is restricted to the loser cells.

742V **Investigating the influence of the transcription factor *hindsight* on a Notch-induced tumor model** Emily D. Baker, Bruce H. ReedBiology, University of Waterloo

Imaginal tissues in *Drosophila* undergo rapid proliferation to replace larval tissue with progenitor cells that will differentiate into adult tissue. Primarily composed of epithelial cells, imaginal rings are precursors to the adult digestive system and are located in the salivary gland, foregut and hindgut. At the posterior of the salivary gland imaginal ring (SGIR) is a transition zone where imaginal ring cells meet gland cells. Transition zones are known tumor hotspots and at this site in the salivary gland, tumors can be induced by the overexpression of Notch (Yang et al., 2019). It is known that the gene *hindsight* plays many diverse roles in the development of *Drosophila* and its protein product has been suggested to participate in a feed-forward loop with Notch to regulate the activity of the M phase inducer, String. Our lab has identified *hindsight* expression in the transition zone of the SGIR and in the anterior-most region of the hindgut imaginal ring. Furthermore, ectopic expression of *hindsight* mitigates Notch-induced tumor development. We are interested in the mechanism by which Hindsight functions in this tumor model and if this aligns with the aforementioned feed-forward loop.

743V **CRISPR/Cas9 Modification of Bloom Syndrome Helicase (Blm) to Assess Regulation of Blm Function** Lahari

Pokala[1], Evan Dewey[2], Jeff Sekelskey[21]North Carolina School of Science and Mathematics, [2]University of North Carolina at Chapel Hill

Bloom syndrome is a rare autosomal recessive genetic disorder causing chromosomal abnormalities including loss of heterozygosity (LOH) and genome instability (Heyer, et al. 2010). The disorder is marked by predisposition to numerous cancers (Hickson, 2003). Recent studies showed deletion of Blm aa 576-720 caused increased chromosome segregation defects, mitotic crossovers, as well as decreased hatch rates. This region will be further explored to determine the impact of predicted phosphorylation residues on regulation of DNA repair and other Blm functions. Assessing roles of phosphorylation with S to A (phospho-dead) and S to D (phospho-mimetic) mutations in the regulation of Blm is important to enhance understanding of tumorigenesis in Bloom syndrome. Goldenbraid cloning will integrate DNA fragments with phosphorylation predicted S to A (phospho-dead) and S to D (phospho-mimetic) residues into Omega 2K plasmids, as well as gRNAs into a PU6K plasmid. These plasmids will be injected into embryos to allow for CRISPR/Cas9 genome editing and subsequent progeny will be studied through DNA repair assays. S to A mutant and gRNA DNA fragments were integrated into respective plasmids. Following embryo injection and genome editing by single-strand annealing, S to A phospho-dead mutations might result in decreased DNA repair efficiency. The successful creation of these plasmids is the preliminary step and current extent of results. Genetic tests are in progress. Recognizing the significance of predicted phosphorylation sites will allow for better understanding of Blm function and DNA damage response in prevention of genome instability and cancer. Continued research may include investigating phosphorylation sites in different Blm regions.

744T **dSmad2 differentially regulates dILP2 and dILP5 in insulin producing and circadian pacemaker cells in adult females** Samuel Goldsmith, Stuart NewfeldSchool of Life Sciences, Arizona State Univ

While much is known about environmental influences on metabolism and systemic insulin levels, less is known about how those influences are translated into molecular mechanisms regulating insulin production. To better understand insulin regulation we generated marked cells homozygous for a null mutation in the TGF-β signal transducer dSmad2 in unmated adult females. We then conducted side-by-side single cell comparisons of dILP2 and dILP5 pixel intensity in dSmad2- mutant and wild type cells. The analysis revealed three previously unknown features of dSmad2 regulation of insulin. First, we discovered that dILP5 is expressed and regulated by dSmad2 outside insulin producing cells (IPCs) in circadian pacemaker cells (CPCs). Second, regulation by dSmad2 differs between dILP2 and dILP5 within IPCs. Third, regulation differs for dILP5 between IPCs and CPCs. In dSmad2 mutant IPCs, dILP2 is increased and dILP5 is present but unaffected. In contrast in dSmad2 mutant CPCs, dILP2 is not present and dILP5 is decreased. Further studies showed that modes of dSmad2 regulation differ between dILP2 and dILP5. dSmad2 antagonism of dILP2 in IPCs is robust but dSmad2 regulation of dILP5 in IPCs and CPCs toggles between antagonism and agonism depending upon dSmad2 dosage. Companion studies of dILP2 and dILP5 in the IPCS of upd2 mutant unmated adult females showed no changes. Taken together, single cell studies reveal that mechanisms of dSmad2 regulation of dILP2 and dILP5 are context dependent and that in adult females dSmad2 acts independently of upd2.

745T **Insulin/insulin-like growth factor signaling pathway promotes increased body fat in *Drosophila* female** Puja Biswas, Colin Miller, Elizabeth RideoutCellular and Physiological Sciences, University of British Columbia

In many animals, including *Drosophila*, females store more body fat than males. One gene that contributes to this sex difference in *Drosophila* body fat is *brummer* (*bmm*), a highly conserved triglyceride lipase. Normally, *bmm* mRNA levels are higher in males than in females. This male bias in *bmm* mRNA levels contributes to the sex difference in body fat, as loss of *bmm* largely eliminates the sex difference in body fat. Yet, it remains unclear which factors regulate the sex difference in *bmm* mRNA levels. One widely recognized regulator of *bmm* mRNA levels is the insulin/insulin-like growth factor signaling pathway (IIS), where high levels of IIS activity repress *bmm* mRNA levels. When we monitored *Drosophila* insulin-like peptides (*Dilps*) and IIS activity in adult flies, females had increased mRNA levels of multiple *Dilps* than males, and higher IIS activity. Females also showed higher peripheral insulin sensitivity than males. We next asked whether higher IIS pathway function in females contributed to their ability to store more body fat than males. To test this, we used inducible gene expression systems to either ablate or activate the insulin producing cells (IPCs), a key source of circulating Dilps, in adult flies shortly after eclosion. Loss of IPCs in females significantly reduced body fat, with no effect in males. We replicated this female-specific effect on body fat using RNAi to reduce Dilp levels in the IPCs. Increased IPC activity, on the other hand, augmented body fat in males with no effect in females. Together, these data suggest that the sex difference in IIS function contributes to the male-female difference in body fat. While future experiments are needed to test whether sex-specific IIS regulation contributes to male-biased *bmm* mRNA levels, and whether the effects

of IIS on body fat are mediated by *bmm*, our data demonstrate sex differences at multiple levels of IIS regulation that contribute to a male-female difference in pathway function.

746T **Neurodevelopmental Effects of Parental High Sugar Diet on D. melanogaster Progeny** Nina Brown, Manaswini Sarangi, carina Yiu, Monica DusUniversity of Michigan

Parental consumption of diets high in saturated fat and refined sugar and high body mass index are associated with a higher incidence of neurodevelopmental conditions. The molecular, cellular, and neural mechanisms that underlie these phenomena have been hard to define due to the complexity of development and the difficulty in uncoupling the parental and fetal nutrient environment during development. To overcome these limitations we have been using the fruit fly D. melanogaster, where ~~neuro~~development occurs in the "closed system" of the embryo after fertilization. Our metabolomics studies show that progeny from high sugar-diet fed parents develop in a nutrient scarce environment, which is reflected in developmental and metabolic phenotypes in these animals. Our current research is aimed at identifying the physiological, molecular, and cellular mechanisms~~,~~ underlying the observed phenotypes in lean/fat mass differences, fecundity, and life span differences amongst adult ~~flies~~ progeny from high sugar diet fed parents.

747T **The Interplay of peroxisome and mitochondrial dynamics during aging in *Drosophila melanogaster*** Ankur Kumar[1], Hua Bai[21]Genetics Development and Cell Biology, Iowa State University, [2]Genetics, Development and Cell Biology (GDCB), Iowa State University

Damaged mitochondria are repaired and recycled through the mechanisms of mitochondrial dynamics in response to stress; this helps in restoring cellular homeostasis. Mitochondrial dynamics have emerged as a novel regulator of aging in recent years. During aging, alterations in mitochondrial morphology and structure have been observed. Researchers have performed genetic manipulations of genes involved in the fission and fusion of mitochondria, which extended the lifespan. However, the causes of the age-dependent alteration in mitochondrial dynamics remain unanswered. Our focus is to explore the involvement of the peroxisome in maintaining mitochondrial homeostasis during animal aging. Recent studies in our lab have shown mitochondrial morphology alteration due to impaired peroxisomal protein import in aging oenocytes (hepatocytes) of fruit flies. We found an increase in mitochondrial size in oenocytes during fly aging. Similarly, we have found that the knockdown of Pex5, a peroxisomal import protein, alters mitochondrial morphology. Interestingly, we also have found that peroxisomal plasmalogen level decreases in aging flies and knocking down the genes involved in plasmalogen synthesis, such as GNPAT (Glyceronephosphate O-acyltransferase) resulted in enlarged mitochondria. Our newly developed live tissue imaging technique in adult flies also have shown the similar alterations. Now, our further goal is to identify the interaction between plasmalogens and mitochondrial fission machinery.

748T **Mef2 and Gga regulate lifespan by interacting with sex-specific heat shock proteins in post-mitotic neurons** Jacquelyn Yarman, Rui Sousa-Neves, Claudia Mieko MizutaniCase Western Reserve University

Circumstantial evidence shows that lifespan is a genetically controlled, species-specific trait. Despite significant research advances associating metabolic pathways, nutrient sensing, and stress response with aging, our understanding of the cellular mechanisms that regulate lifespan is still limited. To better understand the processes that control aging, we focused on two closely related species that have differential stress response and lifespan, *Drosophila melanogaster* and *Drosophila simulans*. The evolutionary proximity of these species allows for obtaining hybrids between them to test if there are genes on the X chromosome that regulate lifespan. Here we show that *D. simulans/D. melanogaster* male hybrids with an X chromosome from *D. simulans* live significantly longer than either pure species, indicating there are indeed X-linked alleles that regulate longevity. In contrast, female hybrids with X chromosomes from both species live much shorter lifespans than each pure species and male hybrids, indicating that the longevity alleles on the X chromosome are recessive. To identify relevant genes in the observed dysregulation of hybrid lifespan, we searched for genes divergent between the parental species. Since there is evidence that brain aging impacts systemic aging, we focused on divergent genes potentially involved in brain homeostasis. Next, we tested if the loss of function of these genes by RNA interference (RNAi) in post-mitotic neurons modified longevity in *D. melanogaster*. Here we show that when aged at 25°C, males expressing either RNAis against *Myocyte enhancer factor 2* (*Mef2*) or *Golgi-localized, γ-adaptin ear containing, ARF binding protein* (*Gga*) have increased lifespan. In contrast, like in hybrids, females expressing these RNAis do not exhibit extended lifespan. These effects are more prevalent when flies are aged at 29°C, indicating that lifespan regulation by Mef2 and Gga is sex and temperature dependent. Next, we searched for sex-specific heat shock proteins (HSP) that physically interact with Mef2 and Gga to identify HSP70Bb and HSP68. Here we show that when sex-specific HSP70Bb or HSP68 and Gga are knocked down in post-mitotic neurons at 29°C, males no longer exhibit increased

lifespan. Overall, our results show that systemic lifespan regulation is sex and temperature dependent via neurons that express a transcription factor required for neuronal maintenance and a protein that mediates post-Golgi vesicle formation.

749T ***PWP1 mediates intestinal stem cell homeostasis in a nutrient dependent manner and affects aging*** Gaia Fabris[1,2], Arto Viitanen[1,2], Ville Hietakangas[1,21]Faculty of Biological and Environmental Sciences, University of Helsinki, [2]Institute of Biotechnology, University of Helsinki

One of the hallmarks of aging is the loss of regenerative capacity and homeostasis in tissues with high cellular turnover[1,2], where integrity is maintained by self-renewal and differentiation of resident stem cells. Importantly, the ability to replace damaged cells is gradually reduced as stem cells age[1,2] and nutrition appears to have a prominent effect on the maintenance of their functions[3]. The intestinal epithelium is an optimal model for studying stem cell nutrient regulation, as it is characterized by high cellular turnover and remarkable plasticity in response to nutrient intake[4].

The nutrient-dependent mTOR signalling pathway is known to be activated in intestinal stem cells (ISCs) during regenerative growth[5], and unpublished work from our lab has shown that nutrient-induced mTORC1 activation contributes to ISC differentiation. This highlights the pivotal role this pathway might exert in the regulation of intestinal homeostasis in response to nutrients. Previous research from the lab discovered a nutrient-dependent role for Periodic tryptophan protein 1 (PWP1) downstream of the nutrient-responsive Insulin/mTOR signalling pathway in *Drosophila* fat body [6]. PWP1 is a chromatin-binding protein implicated in germline stem cell regulation[7], but its role in somatic stem cells and aging is so far unknown.

My research focuses on exploring the regulation and function of PWP1 in intestinal epithelium homeostasis. I have found that PWP1 is expressed in ISCs in a region-specific manner and its subcellular localization is strongly nutrient-responsive. My data also shows that PWP1 has a nutrient-dependent role in ISC regulation, affecting both cell number and cellular volume. Furthermore, the genetic modulation of PWP1 in the ISC-derived cells prominently impacts intestinal epithelium during aging and organismal lifespan. Altogether, my work highlights a new role for PWP1 in the context of nutrient regulation of ISCs and will contribute to a better understanding of the relationship between nutrient availability, cellular homeostasis and aging.

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 101028177 750T **Topoisomerase 3b enhances stability of maternal mRNAs that are essential for neurodevelopment of progeny** Seung Kyu Lee, Aaron Park, Shuaikun Su, Tianyi Zhang, Yutong Xue, Weiping Shen, Weidong WangNational 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 or mRNA levels remain unclear.

During the quiescent period of ovary development, the oocyte remains transcriptionally inactive, yet preserves

translation of stored mRNAs. In *Drosophila*, oocyte development can be arrested at the quiescent state by restricting nutrition and mating, which provides an ideal environment for studying mRNA decay and translation without transcription. Using this model, it has been reported that FMRP enhances translation of mRNAs that are involved in neurodevelopment and mental health. Here, we used the quiescent oocyte model to examine if TOP3B-TDRD3 promotes mRNA translation or degradation in oocytes, and whether this maternal function of TOP3B contributes to zygotic neurodevelopment. We found that the embryos derived from the *Top3b*$^{-/-}$ or *Tdrd3*$^{-/-}$ oocytes under normal nutrition conditions develop normally. Conversely, when mothers were grown in nutrient-restricted conditions (translation inhibition) in oocytes, the embryos derived from *Top3b*$^{-/-}$ or *Tdrd3*$^{-/-}$ oocytes exhibit infertility and abnormal neurodevelopment, including severe defects in the ventral nerve cord (VNC) formation. These phenotypes resemble those observed in the embryo from *fmr1*^{RNAi} flies, suggesting that the TOP3B-TDRD3 complex has a similar function as FMRP in promoting zygotic neurodevelopment. Our RNA-seq analysis showed that a subset of mRNAs undergoes increased degradation in translation-inhibited *Top3b*$^{-/-}$ oocytes, suggesting that these mRNAs may depend on TOP3B for their stability. These TOP3B-regulated mRNAs overlap with those regulated by FMRP, suggesting that TOP3B and FMRP may work coordinately to regulate mRNA degradation. Ribo-seq data showed that translation of the TOP3B-regulated mRNAs is not significantly altered in *Top3b*$^{-/-}$ oocytes. Together, our findings support a model that the TOP3B-TDRD3 complex works with FMRP to regulate mRNA degradation in oocytes, and this function is critical for normal neurodevelopment in embryos.

751T **Characterization of DNA Repair Function in Dna2 Mutants** Sabah Shammari[1], Hamiya Sohail[1], Elyse Bolterstein[2], Sarah Alfaqih[21]Biology, Northeastern Illinois University, [2]Northeastern Illinois University

DNA damage can cause mutations that lead to cancer and disease in many organisms. *Drosophila* is an excellent model for studying DNA repair mechanisms because it contains orthologs for many DNA repair genes. An ortholog of the repair and replication gene *DNA2* has been recently identified as *mus109* in Drosophila. There are three available alleles of *Dna2* (*Dna2*^{D1}, *Dna2*^{D2}, and *Dna2*^{IS}) that have been shown to have sensitivity of MMS and ionizing radiation. However the repair function of Dna2 has not yet been fully characterized. To identify the pathways associated with Dna2, we treated combinations of mutant alleles (*Dna2*^{D1}/*Dna2*^{IS} and *Dna2*^{D2}/*Dna2*^{IS}) with mutagens that stress various replication and repair processes. We found that *Dna2* mutant flies were sensitive to hydroxyurea and topotecan, suggesting that Dna2 is important in responding to replication stress. In contrast, *Dna2* mutants were not sensitive to potassium bromate (oxidative stress) or bleomycin (radiomimetic, double-strand breaks), suggesting that Dna2 is not critical in those repair processes. We also tested lifespan of our *Dna2* mutants and found that the mutation had little effect on lifespan compared to *w*^{1118} wild type controls. We hypothesize that differences in mutagen sensitivity between the *Dna2*^{D1} and *Dna2*^{D2} alleles can be attributed to differences in mutant protein functionality, as the *Dna2*^{D2} mutation occurs downstream of the *Dna2*^{D1} mutation, which may allow for more functional protein to be translated. Together these results provide researchers with the fundamental understanding they need to better explore how DNA repair pathways related to aging and disease.

752T **Experimental Evolution to identify genes that contribute to fitness in high-sugar-fed Drosophila melanogaster** Thomas Rundell[1], Azva Alvi[1], Melina Bruneli[1], Gabrielle Safian[1], Laura Musselman[21]Binghamton University, [2]Biology, Binghamton University

Evolve-and-resequence approaches have been shown to dramatically alter both phenotypic and genotypic characteristics of populations under laboratory selective pressures. In this project, an outbred population made from wild-caught Drosophila was subjected to a control or high-sugar (HS) feeding paradigm for many generations. HS feeding reduces both the lifespan and healthspan in adult Drosophila. Sexes were separated and aged on either diet until mid-life, then mated to produce the next generation, allowing selection for protective alleles. Alleles that increase survival, metabolic homeostasis, and fecundity are hypothesized to be favored under the HS diet. We found that all selected populations increased their lifespan and healthspan over time. Four control and four HS-selected populations have been compared using pooled DNA sequencing coupled with RNA sequencing to identify specific, enriched loci that may have conferred protection against the negative sequelae of caloric excess. One cohort of genes identified across multiple HS-selected populations contained acetylcholine related genes including ChAT, CHT, and mAChR-m. We have used genetic approaches to identify a novel link between acetylcholine signaling and lifespan on a high-sugar diet.

753T **Obesity Is Not A Direct Cause Of Infertility** Rodrigo Dutra Nunes, Daniela Drummond-BarbosaGenetics Department, University of Wisconsin-Madison

Obesity correlates with infertility through poorly understood mechanisms. Previous studies showed that *Drosophila* females with high sugar diet (HSD)-induced obesity produce fewer eggs. We analyzed oogenesis in these HSD obese females, finding increased death of early germline cysts and vitellogenic follicles. We next asked if obesity itself causes reduced fertility using genetically obese females (adult adipocyte-specific RNAi against anti-obesity genes *brummer* or *adipose*), which have increased triglyceride content and larger adipocyte lipid droplets (comparable to those of females on a HSD). Strikingly, these genetically obese females had no changes in oogenesis/fertility relative to control RNAi females, indicating that obesity *per se* is not sufficient to reduce fertility. HSD obese (but not genetically obese) females had increased levels of glucose, trehalose, glycogen, and insulin resistance. A previous study showed that dietary water can revert the reduced longevity of HSD obese females. We therefore tested if "dehydration" might contribute to the oogenesis defects on a HSD. Indeed, dietary water supplementation restored normal egg production to HSD obese females. Notably, these females remained obese with high glycogen, trehalose, and insulin resistance levels, but glucose levels were drastically reduced. Altogether, our data show that obesity, insulin resistance, and high glycogen are not causally linked to the reduced fertility of HSD obese females. Instead, there is a strong correlation between high glucose levels and reduced egg production. Future studies will address whether high glucose or other dietary water-dependent factors are causally linked to the lower fertility of HSD obese females.

754T **Effects of neural factors on aging-related muscle degeneration in the fly model** Selma Atic, Lilla McLendon, Christina Talley, Anton BryantsevKennesaw State University

Striated muscles make a conservative tissue that enables locomotion and supports organismal functions in various other ways. Muscle tissue tends to decline with age, which is a universal phenomenon observed across a wide range of species. In elderly humans, exacerbated muscle wasting (known as sarcopenia) is associated with increased morbidity and mortality. The genetics of aging-related muscle decline is not fully understood, partially because of the lack of a comprehensive list of genetic factors that can influence muscle aging. To improve screening efforts to identify novel "sarcopenia genes", our laboratory has developed a method for quantitative assessment of muscle degeneration using the jump muscle as a model. Based on preliminary results, we hypothesized that the rate of muscle degeneration in aging flies could be affected by mutations causing neurophysiological phenotypes. In this study, we demonstrate that flies that are genetically homozygous for hyperactive alleles (e.g. *Syn[97]*, *Ih[f03355]*) have statistically higher muscle degeneration rates than their heterozygous controls. From the other hand, a complete functional blockade of the jump muscle from neurogenic stimulation does not prevent spontaneous degeneration of its muscle fibers upon aging. Our findings suggest that aging-related muscle degeneration is a stochastic process that can be caused by intrinsic muscle factors but is greatly influenced by the activity of the nervous system. Our data highlight the importance of neural factors in muscle aging.

755T **Investigating the role for diacylglycerol in heat tolerance in *Drosophila melanogaster*** Sunayn A Cheku[1], Blase Rokusek[1], Sunanda Rajput[1], Lawrence G. Harshman[2], Kimberly A. Carlson[11]Biology, University of Nebraska at Kearney, [2]Biological Sciences, University of Nebraska-Lincoln

Thermal tolerance in *Drosophila* has become increasingly relevant given the expected increase in global temperatures due to climate change. The investigation of DAG in heat tolerance is one of significant interest considering previous lipidomic studies within the context of thermal regulation. Evidence from a study by Ko and colleagues (2019) showed that lines selected for elevated temperature knockdown resistance had a statistically significant increase in diacylglycerol (DAG) levels. This exploratory project hypothesized flies administered DAG would show increased heat tolerance.
To verify the heat tolerance assay, flies were heat hardened at 37°C for 1 hour in a heat tolerance apparatus that consisted of a board to clip on individual tubes, a tank filled with water, and an immersion circulating heater to maintain temperature, before being tested 6h or 24h later. The assay consistently differentiated hardened and control flies, regardless of sex or temperature ($p \leq 0.05$). The experimental parameters for this project were extensive. Experimental groups included sex (female or male); temperature (39°C and 40.5°C), exposure time (6h, 12h, 24h, and 3/4d), anesthesia type (chloroform, ether, or CO_2), and route of DAG administration (transdermal, subcutaneous injection, and oral). Time to knockdown was measured by manual observation. Flies receiving either a DAG/PEG oral suspension compared to a PEG control demonstrated a trend for better heat tolerance in males at 40.5°C and in females at 39.5°C both at 24h post treatment, although not statistically significant ($p \geq 0.06$-0.1), they did approach significance with subsequent trials. Notably, at 6h post-treatment, these groups demonstrated significantly worse heat tolerance than controls ($p \leq 0.0005$). Due to the inconsistent results, currently it is not conclusive that DAG has an effect or not on heat tolerance, at least within the bounds of our experimental setup, which was extensive. The significance of this exploratory study was the multiple parameters tested, but the limitation may be that the parameters tested were not responsive to the assays

performed. While the results are inconclusive, they can inform future research investigating the molecular mechanisms of heat tolerance in *Drosophila*. The project described was supported by grants from the National Institute for General Medical Science (GM103427 & 1U54GM115458).

756T **Proteome-wide Quantitative Analysis of Redox Cysteine Availability in the Drosophila Eye Reveals Oxidation of Phototransduction Machinery During Blue Light Exposure and Age.** Sarah Stanhope, Vikki WeakeBiochemistry, Purdue University

During oxidative stress, reactive oxygen species (ROS) can modify and damage cellular proteins. In particular, the thiol groups of cysteine residues can undergo reversible or irreversible oxidative post-translational modifications (PTMs). Identifying the redox-sensitive cysteines on a proteome-wide scale can provide insight into those proteins that act as redox sensors or become irreversibly damaged upon exposure to oxidative stress. To identify redox-sensitive cysteines, we compared two mass-spectrometry based labeling approaches using *Drosophila* S2 cells—Stable Isotope Cysteine Labeling with Iodoacetamide (SICyLIA) and isobaric label sixplex reagents (iodo-TMT). We showed that the iodo-TMT approach was more effective than SICyLIA, identifying more than twice as many oxidized and/or reduced peptides. We next profiled the redox proteome of the *Drosophila* eye under oxidative stress from either prolonged blue light or aging. In the eye, both blue light and aging cause a significant increase of oxidative stress. We found that there is oxidation of essential phototransduction machinery such as inactivation no afterpotential C (inaC), inactivation no afterpotential D (inaD), no receptor potential A (norpA), and retinal degeneration A (rdgA) during blue light exposure and aging. Interestingly, the identified oxidized cysteine residues are different between redox datasets, indicating that blue light- and age-associated oxidative stress impact the proteins differently. Additionally in the blue light redox dataset, we showed that enzymes in methionine metabolism such as adenosylhomocysteinase (ahcy) were irreversibly oxidized at critical residues required for protein activity. Ahcy is essential for methionine metabolism because it is the only enzyme that catabolizes S-adenosylhomocysteine (SAH), which is produced as a byproduct of cellular methylation reactions. Under blue light exposure and aging, we observed a significant increase in the abundance of SAH suggesting that methylation reactions in the cell could be impacted by changes in redox status via alterations in methionine metabolism. These data provide a rich resource for researchers interested in how oxidative stress impacts the redox proteome, and to our knowledge – provide the first analysis of the redox proteome of either S2 cells or *Drosophila* eyes.

757T **Investigating the role of Glycerol-3-phosphate dehydrogenase 1 (GPDH1) in *Drosophila* growth and development.** Shefali A Shefali, Madhulika Rai, Sarah Carter, Nader Mahmoudzadeh, Hongde Li, Maria Sterrett, Jason M TennessenBiology, Indiana University Bloomington

As the fruit fly, *Drosophila melanogaster*, progresses from one life stage to the next, many of the enzymes that compose intermediary metabolism undergo substantial changes in both expression and activity. These predictable shifts in metabolic flux allow the fly to meet stage-specific requirements for energy production and biosynthesis. In this regard, the enzyme Glycerol-3-phosphate dehydrogenase (GPDH1) was the focus of biochemical genetics studies for several decades, and as a result, is one of the most well characterized enzymes within *Drosophila* metabolism. Among the findings from these earlier studies is that, GPDH1 promotes mitochondrial energy production and glycerol biosynthesis, while also serving a key role in maintaining cellular redox balance. To extend these studies and understand the importance of GPDH1 in larval development, we are examining the tissue-specific expression of this enzyme. Our preliminary analysis confirms previous studies reporting that GPDH1 is most abundantly expressed in the fat body and that its expression is regulated in a stage-specific manner. However, we unexpectedly found that GPDH1 expression within the fat body undergoes a significant decline during the mid-L3 stage. Moreover, our studies also reveal that GPDH1 expression within the fat body is sensitive to dietary nutrition and disruption of metabolic regulatory networks, raising the possibility that this enzyme plays an essential role in coordinating developmental progression with nutrient availability. Supporting this hypothesis, our preliminary studies suggest that GPDH1 influences larval growth by controlling the abundance of dihydroxyacetone phosphate. Based on these findings, we are now using a combination of metabolomics and *Drosophila* genetics to understand how GPDH1 can influence larval growth by regulating the abundance of glycolytic intermediates.

758T **Investigating the function of sleep in *Drosophila melanogaster*** Samantha J Tener[1], Jungwon Lee[2], Kairaluchi Oraedu[3], Carly Y Lam[3], Jared A Gatto[3], Mimi Shirasu-Hiza[31]Genetics and Development, Columbia University, [2]Hamilton College, [3]Columbia University

Sleep is an evolutionarily conserved behavior across the animal kingdom, yet the physiological functions of sleep are

unknown. In humans and model organisms, a chronic lack of sleep leads to illness and death; thus, sleep must promote a core function of health. Our lab previously identified a bidirectional relationship between sleep and oxidative stress, supporting sleep's role as an antioxidant. In this study, we sought to identify the molecular mechanism(s) by which sleep defends against oxidative stress. Through an RNA-sequencing experiment, we found differential expression of metabolism-related genes, suggesting that metabolism links sleep to oxidative stress. In addition to an increased sensitivity to oxidative stress, we found that short-sleeping flies also share an increased sensitivity to starvation. Taken together, we are working to discover whether sleep functions through the same or separate mechanisms to promote both metabolic and oxidative stress homeostasis, starting with a metabolic characterization of short-sleeping flies compared to their controls.

759T **Ameliorating the effects of an environmental toxin in a *Drosophila* model of Parkinson's Disease** Dionne Williams, Hakeem LawalDelaware State University

Parkinson's Disease (PD) is a neurodegenerative disorder characterized in part by the selective loss of dopaminergic neurons in the substantia nigra pars compacta. Although the precise cause of PD is not yet fully understood, environmental factors are known to contribute to the etiology of a vast number of cases. Rotenone, a pesticide that inhibits Complex 1 of the mitochondrial Electron Transport Chain, is one such toxin. Importantly, there is no known cure for PD and effective treatment options are severely limited both in number and efficacy. We are interested in developing neuroprotective strategies that may lead to more effective treatments for the disease. This project studies the effects of rotenone-induced toxicity in adult *Drosophila melanogaster* and the neuroprotective capacity of dacarbazine, a possible anti-PD drug that was identified in a previous pharmacological screen. We hypothesized that dacarbazine will confer both organismal and neuroprotection against rotenone-induced toxicity and mitochondrial dysfunction. And we report that treatment with dacarbazine led to a partial rescue of organismal lethality induced by rotenone. Further, we measured the effect of rotenone on mitochondrial oxygen consumption rate (OCR) using the Seahorse Analyzer and tested whether treatment with dacarbazine can ameliorate the effects of the rotenone inhibition of the mitochondria, and we present preliminary data on the effect of dacarbazine on OCR in adult *Drosophila melanogaster* brains and in Schneider (S2) cells. In addition, we utilized an immunohistochemistry technique to count the dopaminergic neurons in adult *Drosophila* brains to further investigate the potential neuroprotective effects of dacarbazine in our *Drosophila* model of PD. In sum, our report shows the utility of a potential neuroprotective chemical against a model of PD and suggests a possible neuroprotective mechanism against the diseases.

760T **The role of Spenito and sex determination in establishing sexually dimorphic metabolism** Arely V. Diaz, Tânia ReisDepartment of Medicine, University of Colorado School of Medicine

Metabolism in males and females is fundamentally different. Previously, we discovered that Spenito (Nito), an RNA-binding protein and regulator of sex determination, is required for proper fat storage. Through its m^6A RNA modification role, Nito is essential for splicing of sex determination genes and consequently for proper sex establishment. At the larval stage males have significantly higher levels of fat than females. We found metabolic genes with sexually dimorphic expression in the larval fat body (FB). We propose a role for Nito in the establishment of metabolic sexual dimorphisms and maintenance of differential gene expression. Larval FB-specific Nito knockdown recapitulated our previously reported lean phenotype. Additionally, we also observe abolished fat differences between males and females in larvae lacking Nito in their fat cells. To get initial insight into a potential dimorphic mechanism, we analyzed the transcriptional profiles of the larval FB in males versus females and observe differential expression of several metabolic genes, including lipases such as Yp2 and Yp3. We further show that Nito is required for regulating the sex-dimorphic expression of these lipases and this misregulation correlates with the lack of male versus female fat differences. To address the role of the sex determination pathway and male versus female physiology in metabolic sexual dimorphisms at the larval stage, we overexpressed Tra specifically in the FB. Tra overexpression in the FB 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, Tra overexpression shifted the male FB expression of Yp2 and Yp3 to a female-like state. Altogether, our findings support a model in which Nito regulates metabolic sexual dimorphisms and differential expression of target genes partially through its regulation of the sex determination pathway.

761T **Metabolic regulation of protein degradation by N-terminal acetylation controls germline stem cell differentiation** Bruno Hudry, Charlotte FrancoisInstitut de Biologie Valrose, Université Côte d'Azur, CNRS, Inserm

The molecular mechanisms that connect cellular metabolism and cell fate decisions, as cells differentiate, remain

incompletely understood. Here, we use *Drosophila melanogaster* spermatogenesis to investigate how metabolic signals contribute to stem cell differentiation and germline homeostasis. We discovered that external citrate supply fuels Acetyl-CoA production and is essential for the commitment to the final stage of germline differentiation. In contrast to known metabolic control of gene regulation, high Acetyl-CoA level promotes NatB-dependent N-terminal protein acetylation. Genetic and biochemistry experiments establish that the critical role of N-terminal acetylation is shielding proteins from proteasomal degradation by the specific ubiquitin ligase, Ubr1. The break in differentiation induced by NatB targets destabilization is reversible. Our work uncovers that a protein post-translational modification couples the dynamics of stem cell differentiation to the metabolic state, revealing that N-terminal protein acetylation is physiologically essential for animal germline homeostasis. The instructive role of circulating citrate in controlling cell functions through protein stability might be significant in more biological contexts.

762T **Expression of the alternative oxidase reconfigures the mitochondrial electron transfer system, promoting thermogenesis and increased biomass in *Drosophila* larvae** Geovana S Garcia, Marcos T OliveiraDepartment of Biotechnology, Sao Paulo State University (Jaboticabal campus)

The alternative oxidase (AOX) is a terminal oxidase naturally present in the mitochondrial electron transfer system (ETS) of most organisms, but absent in insects and vertebrates. It oxidizes coenzyme Q reduced by one of the mitochondrial inner membrane-embedded dehydrogenases and reduces O_2 in H_2O. AOX then creates an extra pathway for O_2 reduction, which is particularly important when the cytochrome c segment of the ETS is overloaded, relieving oxidative stress. Notably, diverse phenotypes related to mitochondrial diseases have been attenuated by its xenotopic expression in animal models. However, its non-proton-pumping nature may uncouple mitochondria, generating excess metabolic heat, which may interfere with animal metabolism and physiology. We have previously shown that *Drosophila melanogaster* lines constitutively expressing AOX from *Ciona intestinalis* (Tunicata: Ascidiacea) are cold stress resistant. To investigate whether that is caused by thermogenesis, here we explored larval metabolism under 25 and 12°C, a particularly stressful temperature. Interestingly, AOX-expressing larvae are up to 70% more active, independently of temperature. They also lose body heat less pronouncedly, maintaining their body temperature ~0.2°C higher than that of control larvae. AOX-expressing larvae also have an increased body mass, associated with an increased fat accumulation, being ~33% higher than that of controls at 12°C. Mitochondrial physiology analyses indicate that AOX increases leak respiration, decreasing ~30% oxidative phosphorylation (OXPHOS) efficiency via the non-proton-pumping glycerol-3-phophate dehydrogenase (mGPDH). On the other hand, complex I (CI)-driven respiration is ~30% increased and the ETS activity is significantly less limited by the ATP synthase at 12°C. Our data suggests that AOX and mGPDH functionally interact, dissipating all electron transfer energy as heat, and that this interaction is key to the ETS reconfiguration we observe. As a result of this uncoupling, electron flux driven by CI is increased, perhaps as a compensatory mechanism, consequently stimulating the reactions of the tricarboxylic acid cycle, increasing cataplerosis, and reinforcing the metabolic program of the proliferative tissues of the growing larva. In summary, a combination of increased uncoupled mGPDH-linked respiration and increased cataplerosis contributes to thermogenesis in AOX-expressing larvae, promoting growth, largely under severe cold stress.

763T **Genetic Variation in Dietary Sugar Consumption in *Drosophila*** Mubaraq Opoola, Lucas Fitzgerald, Nicholas Wright, Dae-Sung HwangboBiology, University of Louisville

Sugar is a key part of the daily diet; its overconsumption is often a major contributing factor to many metabolic diseases such as diabetes and cancer. Additionally, some non-metabolic diseases like high blood pressure have been impacted by high sugar consumption. In mammals, prolonged ingestion of excessive dietary sugar promotes overconsumption of the diet partially due to a desensitization of sweetness over time. Yet, its genetic and neuronal mechanisms are not fully understood. Using the fruit fly, *D. melanogaster* as a model system, we investigated the genetic variability of the trait (sugar-induced overfeeding) among eight different wild-type populations and in ~ 170 isogenic lines from the Drosophila Genomic Reference Panel (DGRP). Surprisingly, none of these lines showed an increased food consumption on a high-sugar diet (20% sucrose). This observation suggests that flies employ protective mechanisms from overconsumption of food caused by excessive sugar. A follow-up genome-wide association study (GWAS) using the data from DGRP lines identified ~ 60 genes that may have protective roles from over-consumption of a high-sugar diet. RNA interference (RNAi) mediated genetic screening of these genes showed possible candidate genes affecting sugar-based consumption in Drosophila.

764T **The Integration Institute: Sex, Aging, Genomics, and Evolution (IISAGE)** Nicole C Riddle[1], Peggy Biga[1], Anne Bronikowski[2], Ellie Duan[3], Tony Gamble[4], Erica Larschan[5], Richard Meisel[6], Ritambhara Singh[7], James Walters[8], Ashley

Webb[5], Gerald Wilkinson[91]University of Alabama at Birmingham, [2]Michigan State University, [3]Department of Animal Science, Cornell University, [4]Department of Biological Sciences, Marquette University, [5]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, [6]Department of Biology and Biochemistry, University of Houston, [7]Department of Computer Science, Brown University, [8]Department of Ecology & Evolutionary Biology, University of Kansas, [9]Department of Biology, 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.

765T **Mutations in lamin and how it causes multiple tissue-specific disorders** Bismark Acquah[1], Alysia Vrailas-Mortimer[21]Biological Sciences, Illinois State University, [2]Biochemistry and Biochemistry, Oregon State University

Lamins, the major components of the nuclear lamina where they provide a platform for the binding of proteins to the chromatin and confer mechanical stability (Dittmer and Misteli.,2011). Mutations in the human LMNA gene result in at least 15 distinct disorders ranging from muscular dystrophies to neurological disorders to lipodystrophies **(**Vytopil *et al.*,2003**).** Interestingly, some mutant forms of lamin protein aggregate, which may be toxic to the cells. However, it is unknown how specific mutations in lamin give rise to tissue specific disease. We hypothesize that certain tissues are susceptible to specific lamin mutations due to the inability of tissue specific quality control mechanisms to degrade those mutant forms, leading to protein aggregation and cellular toxicity. I will be testing if tissue specific disease mutations in Lam Dm0, one of the fly homologues of LMNA, cause the protein to aggregate in muscles and/or neurons. We find out that the main forms and the farnesylated forms of the different Lam Dm0 mutant proteins have different expression patterns in the muscles of the flies. In addition, we find that the p38 MAPK (p38Kb) interacts with the CASA complex to regulate the degradation of Lam Dm0, the other fly homologue of LMNA. Future experiments will characterize how these mutant forms of Dm0 affect the functionality of the muscles and neurons in flies and if these forms can be targeted for degradation by p38Kb and the CASA complex.

766T **The impact of modifier genes on obesity and *Drosophila* AKH/glucagon signaling** Audrey Nicol, Rebecca PaluBiological Sciences, Purdue University Fort Wayne

Obesity is a growing concern as 42.3% of people in the U.S were considered obese in the years 2017-2018. Little is known about the genetic components that contribute to weight gain. In humans, the hormone glucagon is a major contributor to the body's energy demand as it helps break down lipids. Therefore, learning more about this pathway could enable a range of therapeutics. In fact, studies have shown that glucagon treatments have helped patients with both weight loss and appetite suppression. In this project, we are analyzing candidate genes that modify the glucagon pathway in *Drosophila melanogaster*. We reduced the expression of the fly version of the glucagon receptor (*AKHR*) in our model. This induces fat retention in the L3 larvae, which mimics obesity in humans. We then crossed our model to the DGRP and look for natural variation in fat content using a density assay. The density assay examines the relative fat levels of the larvae by slowly increasing the amount of sucrose in water. This enables us to observe whether we have lean larvae which float later or fat larvae which float early on. We used the variation in floating concentration to identify candidate modifier genes through GWA. We crossed our *AKHR* RNAi model to RNAi for various candidate modifier genes that may enhance or suppress fat retention. We screened these candidates initially with the same density assay used in the original screen. We further characterized candidates that suppressed or enhanced obesity using biochemical assays to analyze stored metabolites such as triglycerides, glucose, glycogen, and protein. Several candidate genes, including

THADA, *AmyD*, *GluRIIC*, and *CG9826*, significantly impact fat storage in the flies. These have been further analyzed under control, high sugar, and high fat conditions to see if the larvae are resistant to environmental changes. Our goal is to advance our understanding of the glucagon signaling pathway, obesity, and lipid metabolism. We are also hopeful to provide candidate genes that can be regarded as future therapeutic targets.

767T **Investigating the role of PDZD8 in behavior and aging** Leona Hariharan[1], Rajan Thakur[1], Kate O'Connor-Giles[1,21]Department of Neuroscience, Brown University, [2]Carney Institute for Brain Science

The O'Connor-Giles Lab identified PDZD8 in a screen for uncharacterized conserved synaptic genes in Drosophila. PDZD8 is an intrinsic ER transmembrane protein with a synaptotagmin-like mitochondrial lipid-binding proteins (SMP) domain that has been reported to localize to ER-late endosome/lysosome (LE/Lys) and ER-mitochondria membrane contact sites (MCSs). The molecular steps involved in the recruitment of PDZD8 to MCSs have been recently elucidated, yet the in vivo relevance of PDZD8 to neuronal function remains unclear. Interestingly, we find that PDZD8 is expressed at synapses throughout the central nervous system and the larval neuromuscular junction (NMJ), where it localizes to ER-LE/Lys MCSs. PDZD8's expression pattern is consistent with a role in synapse development and function. We have found that activity-induced synaptic growth and neurotransmission are dysregulated in PDZD8 mutants. We further found that PDZD8 positively regulates autophagy to promote synaptic growth. To study the impact of reduced autophagy in the context of aging in PDZD8 nulls, I have used behavior paradigms like the climbing assay and larval crawling to study age-dependent changes in motor activity. I find that loss of PDZD8 results in severe climbing deficits that are exacerbated by age compared to wild-type controls. Interestingly, reduced autophagy with age is known to cause accumulation and aggregation of undegraded protein leading to neurodegeneration. Using a well-characterized neurodegenerative Amyloid-β (Aβ42) model of Alzheimers, I find neuronal overexpression of Aβ42 also leads to similar age-dependent locomotion defects as loss of PDZD8. I further show that PDZD8 can directly modulate the Amyloid toxicity in the Drosophila model of Alzheimer's. Overall, we propose that PDZD8-mediated ER-LE/Lys membrane interactions may play a protective role in age-dependent neuronal proteostasis.

768F **Impact of Genetic Variation on Obesity in *Drosophila melanogaster* through the AKHR pathway** Nay Maung, Allison N Velie, Malaika Ahmed, Chelsea Fischer, John Garces, Grace Lewis, Shana Newman, Nicholas Molisani, Audrey Nicol, Sophia Petrov, Rebecca PaluBiological Sciences, Purdue University Fort Wayne

Obesity is considered a global health issue, and both environmental and genetic factors contribute to this complex disease. As the latter is less understood, genetic contributions to obesity poses a topic of interest. In humans, obesity can be observed through the suppression of the glucagon signaling pathway. Glucagon is involved in the stimulation of lipase, a key enzyme responsible for lipolysis. Inhibiting this action prevents fat catabolism from occurring, allowing for the induction of obesity. Loss of the adipokinetic hormone receptor (*AKHR)*, the analogue for the glucagon receptor in *Drosophila melanogaster*, also induces obesity. *AKHR* normally induces a response to starvation and is partially responsible for maintaining metabolic homeostasis. Reducing expression of *AKHR* inhibits these responses, particularly triglyceride breakdown. To determine if genetic background influences this phenotype, we are crossing a model of reduced *AKHR* expression with the *Drosophila* Genetic Reference Panel (DGRP). We use RNAi against *AKHR* to reduce its expression specifically in the fat body using the driver *r4-GAL4*. To monitor variation in stored triglycerides and obesity, we use a larval density assay previously described in Reis *et al*. 2010 *PloS Gen* and other publications. As our quantitative readout we used the concentration of sucrose at which half the larvae for each strain floats. We plan to use genome-wide association analysis to identify candidate modifier genes. A preliminary GWA has already been completed allowing for a compilation of several candidate genes which are currently being investigated. The overall goal is to compile data for all of the DGRP strains before running a complete GWA and identifying additional strong candidate modifiers that could be used as therapeutic targets or prognostic predictors.

769F **Impact of Social Interactions on Aging in *Drosophila melanogaster*** Yousef B. Emara[1], Christi M Gendron[2], Scott D Pletcher[21]Molecular & Integrative Physiology, University of Michigan, [2]Molecular and Integrative Physiology, University of Michigan

Past research with model systems has established that aging and aging-related molecular pathways influence how individuals are perceived by the opposite sex, and social interactions with both live and dead conspecifics cause short-term changes in stress resistance and fat storage, as well as long-term effects on mortality. Social interaction is also critical to the physical and mental health of humans, but our mechanistic understanding of the underlying molecules and neurons that mediate the social effects on aging, and vice versa, remains rudimentary. To address this knowledge gap,

we sought to determine whether aging is altered by modifying social behavior in *Drosophila melanogaster*, regardless of normal age-dependent changes, and, if so, to elucidate the neurons and neuropeptides responsible for mediating such effects. First, we characterized the changes and patterns of social interactions in *D. melanogaster* as the flies age to establish a baseline for what constitutes «normal» patterns of social interaction among different cohorts of wild-type flies. Video tracking technology was used to calculate the social distance between flies with accuracy and precision at high speeds. Once a baseline was established, we manipulated targeted neural populations using optogenetic techniques, examining their effects on both behavior and longevity. Our preliminary data identify specific neurons that significantly impact social interactions, affecting fly health (as measured by negative geotaxis) and lifespan. Thus, our work demonstrates the powerful impact social environments have on health and aging and lays the groundwork for the dissection of the neural mechanisms involved.

770F **Phenotypic characterization of adaptation to overnutrition in lab-evolved flies** Utsav Nyachhyon[1], Thomas Rundell[2], Laura Musselman[11]Biology, Binghamton University, [2]Binghamton University

Obesity and type 2 diabetes (T2D) have increased globally in adults and children over the last century due to chronic overnutrition. The association of obesity and T2D with increased infection susceptibility results in an increased risk of recurrent infections that can lead to sepsis and death. Although the dysregulation of metabolism has an effect on the ability of the organism to defend against infection, the detailed interactions between metabolism and immunity are not well understood. The development of complications associated with obesity in Drosophila and the conservation of pathways involved in metabolism and immunity make Drosophila a useful model to study these interactions. We hypothesized that immunity might be affected in flies who had been selected for longevity on a high-sugar diet. We used an evolve-and-resequence approach to study the alleles that underlie increased lifespan during high-sugar feeding. Some of the differentially present SNPs and mRNAs were found in genes that encode proteins with roles in immunity. Therefore, we characterized the immune response of high-sugar-adapted flies through infection with *Pseudomonas aeruginosa*. Other potential mechanisms for adaptation to chronic high-sugar feeding could be increased physical activity, reduced consumption, or the reallocation of excess resources into offspring production. Our goal is to leverage this lab adaptation paradigm to uncover the genes and mechanisms linking longevity and metabolism.

771F **Integrating lipid metabolism, pheromone production and perception by Fruitless and Hepatocyte nuclear factor 4** Jie Sun[1], Wen-Kan Liu[2], Calder Ellsworth[2], Qian Sun[3], Yu-Feng Pan[4], Yi-Chun Huang[2], Wu-Min Deng[21]Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, [2]Tulane University School of Medicine, [3]Louisiana State University, [4]Southeast University

Sexual attraction and perception, governed by separate genetic circuits in different organs, are crucial for mating and reproductive success, yet the mechanisms of how these two aspects are integrated remain unclear. In *Drosophila*, the male-specific isoform of Fruitless (Fru), Fru^{M}, 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 (Fru^{COM}) is necessary for pheromone biosynthesis in hepatocyte-like oenocytes for sexual attraction. Loss of Fru^{COM} 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 *Hepatocyte nuclear factor 4* (*Hnf4*) as a key target of Fru^{COM} 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.

772F **The response to oxygen availability in the transcriptome of *Drosophila melanogaster*** George Kapali, Alexander ShingletonBiological Sciences, University of Illinois at Chicago

In almost all animals, physiologically low oxygen (hypoxia) during development slows growth and reduces adult body size. There are systemic endocrine mechanisms that determine growth under hypoxic conditions; however, they are poorly understood. The steroid hormone ecdysone has been shown to regulate growth and body size in response to hypoxia through a mechanism that also slows growth at low nutrition. Hypoxia and low nutrition increases level of circulating ecdysone and suppresses the insulin/IGF-signaling pathway via increased expression of the insulin-binding protein *Imp-L2*. Here we look at the transcriptional response of hypoxia in Drosophila to understand how low oxygen regulates growth via ecdysone. We used q-PCR to assay the expression of genes involved in ecdysone synthesis, export, import and metabolism under normoxic and hypoxic conditions. Counterintuitively, the transcriptional response to

hypoxia does not include upregulation of ecdysone synthesis genes or downregulation of ecdysone metabolic genes. Intriguingly, we observed an elevation of the ecdysone exporter gene *Atet* under hypoxia. This suggests that the elevation of circulating ecdysone levels and ecdysone signaling under acute hypoxia is not achieved through a simple increase in the transcription of ecdysone synthesis genes, but rather through an increased extracellular export of ecdysone.

773F **New explanations for mating-induced structural and metabolic remodeling of Drosophila mid-gut** Tahmineh Kandelouei[1], Mitchell Lewis[2], Bruce Edgar[11]Oncological Sciences, Huntsman Cancer Institute, University of Utah, [2]Biomedical Engineering, University of Utah

We use Drosophila as a model system. Drosophila mid-gut is similar to the mammalian intestine, has a simple structure, and powerful genetic tools exist for probing molecular genetic mechanisms. We perform genetics tests, including RNA-Seq of whole mid-gut and FACS-sorted progenitor cells, metabolomics, and lipidomics analysis. These experiments help us identify the transcriptionally regulated genes and how mating affects other signaling pathways and physiological processes such as intestinal stem cell (ISC) metabolism, proliferation, lineage symmetry/asymmetry, and gut remodeling.

A recent discovery in *Drosophila* has shown that mating-induced 20-hydroxy ecdysone (20HE), produced by ovaries after mating, stimulates ISC proliferation and gut growth. We aim to examine the mechanisms underlying mating contribution to ISC proliferation and gut remodeling upon mating. Our RNA-Seq data of mated female flies have shown the upregulation of genes involved in protein metabolism both at whole-gut and progenitor cells; these Jonah genes are known as serine proteases involved in in the breakdown of dietary proteins; we followed this with lipidomics; lipidomics data analysis shows enrichment of storage and signaling lipids in line with our RNA-Seq data. Since enterocytes (ECs) are the main source of lipid droplets in the midgut, we hypothesize that gut remodeling upon mating happens through increased lipid droplet storage and membrane expansion in ECs; this suggests that gut remodeling mostly occurs through the increase in the size of EC cells. We also hypothesize that 20HE signaling regulates this gut remodeling upon mating through changes in lipid metabolism in Drosophila mid-gut.

This work will provide lead data to understand better how mating and mating-induced steroid signaling regulate intestinal physiology. Reproduction is energetically costly and requires changes in internal organ molecular functions that may not be fully characterized functionally and mechanistically.

774F **Unexpected nuclear roles for Iron Regulatory Protein 1A (IRP1A) in *Drosophila melanogaster*** Minyi Yan, Kirst King-JonesBiological Sciences, University of Alberta

Iron is a vital trace element for nearly all organisms. Improper regulation of cellular or systemic iron concentrations can lead to severe diseases, including porphyria, hemochromatosis, and anemia. Iron concentrations are tightly controlled by iron regulatory proteins (IRPs) at the cellular level. Vertebrate IRP1 and its fly ortholog IRP1A can switch between an RNA-binding form (apo-IRP1) and an aconitase function (holo-IRP1). Under low cellular iron concentrations, IRP1 assumes the RNA-binding form and binds to iron-responsive elements (IREs) found in specific messenger RNAs (mRNAs) that encode proteins relevant for iron uptake, transport, and storage. Both the aconitase and the RNA-binding form are believed to act in the cytoplasm. However, our lab recently demonstrated that holo-IRP1A is a predominantly nuclear protein in the prothoracic gland (PG) and in the fat body. Most other tissues showed the expected cytosolic localization. Overexpression of a nuclear variant of IRP1A perturbed the expression of many iron-linked genes, whereas the RNA-binding apo-form was unable to do so. Remarkably, we found that holo-IRP1A binds to specific histone proteins, including H2Av, and that this interaction was not dependent on DNA. These unexpected results suggest that IRP1A has hitherto undiscovered roles in cell nuclei, where it appears to regulate cellular iron homeostasis via transcriptional control of specific loci. To further examine this, we conducted CUT & Tag (Cleavage Under Targets and Tagmentation) to identify chromatin loci to which IRP1A binds. Preliminary data indicates that IRP1A transcriptionally regulates genes involved in nucleosome dynamics. Furthermore, we will present TurboID data, which we used to identify nuclear interaction partners of IRP1A to understand how IRP1A regulates gene expression. IRP1 has been studied for more than 30 years, but these new findings indicate that our current understanding of how IRP1 controls iron balance in cells is incomplete. We expect that the study of these new and unexpected aspects of iron biology in *Drosophila* will hold true in vertebrate systems as well.

775F **Physiological response to temperature stress in *Drosophila melanogaster* and *D. pseudoobscura*** Natalia Rivera Rincon[1], Ulku Huma Altindag[1], Rita M Grace[2], Arthur Appel[3], Laurie Stevison[11]Biological Sciences, Auburn University, [2]Auburn University, [3]Biological Sciences, AUBURN UNIVERSITY

Climate change is expected to affect biodiversity at different levels, as individuals, populations, or as ecosystems, by altering environmental cues necessary throughout life and across developmental stages. Increasing temperature is the most ubiquitous impact of climate change, and it has been shown to be strongly related to growth rates, fecundity, and fertility. Further, the degree of these responses has been shown to vary between species or even populations of the same species. Here, we compared physiological changes in thermotolerance*, metabolic rates, and* reproduction *i*n response to temperature stress in two major species of fruit fly, *Drosophila melanogaster* (a cosmopolitan species) and *D. pseudoobscura* (an endemic US species). Species-specific treatment crosses were set up at appropriate temperature ranges control vs high temperature (25-29.5ºC for *D. melanogaster*, 20.5-25ºC for *D. pseudoobscura*) and at two different developmental stages: larval and larval+adult; producing ~6000 individuals total[LS1] . An ANOVA test for each measure [LS2] found significant differences between treatments for both species for *thermo-tolerance* ($p<2x10^{-3}$), O^2 consumption ($p<1x10^{-3}$), CO^2 production ($p<2x10^{-3}$), and survival across developmental stages ($p<1x10^{-3}$). Lastly, the ovaries of both species were dissected at early and late meiotic stages and fluorescently stained with DAPI and TUNEL to assay DNA damage. Individual pictures were analyzed to identify the developmental stages present and compare apoptosis based on stage and treatment. Moreover, the ovaries were used for genomic assays to investigate variations in transcriptional control (qtR-PCR) and chromatin availability (ATAC-seq) in response to heat stress. Results from this study will allow for the comparison of different cellular and physiological responses between species with different geographic distributions, thermal tolerances, and development times. Overall, our study will provide a better understanding of the effects of climate change across various species with similar organismal differences.

776F **The role of insulin signaling in sex differences in gene expression** Nafiul Huda[1], Michelle N Arbeitman[2], Rita M Graze[11]Biological Sciences, Auburn University, [2]Biomedical Sciences, Florida State University

The insulin signaling pathway plays an essential role in nutrient sensing, energy homeostasis, and stress responses. However, it can also contribute to sex dimorphism of many traits through interaction with the sex determination hierarchy. For example, insulin signaling modulates sex dimorphism in body size, locomotion, and stress responses. Our previous work has shown that a large proportion of genes show sex differences in gene expression when insulin signaling is significantly reduced - however, the mode by which regulation downstream of insulin signaling results in sex differential expression in adult flies is unknown. The regulation of sex differences in gene expression could be dial-like, allowing each sex to respond differently to gradual changes in insulin signaling. Alternatively, it could be switch-like, which would produce different thresholds for regulatory responses in each sex. In this study, insulin signaling was reduced to different degrees using genetic perturbation, and the regulation of gene expression in adult males and females was assessed, revealing the modes of regulation of sex differences in expression by insulin signaling.

777F **Exploring potential genetic mechanisms underlying the transmission of ethanol resistance to progeny by adults repeatedly intoxicated with ethanol** Michelle A Bonilla[1], Jocelyn Coreas[1], Merna Massoud[1], Cristian Rodriguez[1], Mariano Loza-Coll[21]Biology, California State University Northridge, [2]Biology, California State University, Northridge

There has been a recent renewed interest in the transmission of acquired traits, particularly those related to tolerance to drugs and environmental toxins. Since tolerance is an accepted pre-requisite and strong predictor of addiction, a firmer understanding of the mechanisms underlying the transmission of tolerance could facilitate novel research avenues of importance in public health. Despite the significant advances made in characterizing the epigenetic and molecular mechanisms that underlie the transmission of acquired traits across species, it is still unclear what genetic pathways may connect acquired metabolic, physiological and neurological traits with the epigenetic modifications in germline that are necessary for heredity.

We have recently reported that parental flies that are intoxicated multiple times with ethanol vapors (once a day, for 10 minutes, over a 2 week period) give rise to progeny that is significantly more resistant to the sedative effects of ethanol, and have since established that such transmission is primarily matrilineal. We are now conducting RT-qPCR measurements to determine if there are any differences in mRNA abundances of genes known to mediate ethanol tolerance and ethanol resistance in flies, including from both ovaries from females repeatedly exposed to ethanol compared to those mock exposed to air, and brains of their respective progeny. Here we present preliminary results for Sirt1, Arf6, Slo, Adh, Pum, Hang, InR, Clic, Crz, CrzR, Dally, DopEcr, Hsp26, KCNQ, Tre1, NPF, Rut, Kra and Aru, and discuss opportunities for future research to explore genetic pathways that may be potentially involved in the transmission of ethanol resistance.

778F **The role of phosphoglycolate phosphatase in serving as a metabolite repair enzyme is conserved in**

Drosophila Jennifer Kennell[1], Isabelle Gerin[2], Guido Bommer[21]Biology, Vassar College, [2]de Duve Institute

Even though metabolic enzymes can be quite specific in their substrate recognition, they are also able to bind to other substrates, sometimes producing toxic side products that must be degraded to allow normal biological functions to continue. Recently a highly conserved enzyme, phosphoglycolate phosphatase (PGP), has been identified as a metabolite repair enzyme in mice, human cells, and yeast, by converting toxic side products of glycolysis and DNA repair (4-P-erythronate, 2-P-L-lactate, and 2-phosphoglycolate, respectively) into non-toxic, de-phosphorylated forms. Based on sequence similarity, we have identified *CG5567* as a potential *PGP* ortholog in *Drosophila*. We have found that flies lacking *CG5567* survive to adulthood, unlike *PGP* mutant mice which die as embryos, but are more sensitive to treatment with ethylene glycol, which causes elevated levels of one of these predicted substrates, 2-phosphoglycolate (2-PG). Indeed metabolomic analysis shows extremely elevated levels of 2-PG in *CG5567* knockout flies treated with ethylene glycol, along with the predicted downstream impacts of 2-PG inhibition of triose phosphate isomerase, phosphoglycerate mutase, and succinate dehydrogenase. In addition, the *CG5567* mutant flies are also more sensitive to oxidative stress, which may be due to the elevated levels of 4-P-erythronate, a pentose phosphate pathway inhibitor, we see in these mutants. Yet, we saw no change in the levels of 2-P-L-lactate. *In vitro* analysis with recombinant CG5567 confirms these *in vivo* findings. Together, our results provide strong evidence that *CG5567* is a *PGP* ortholog and that the role of PGP as a metabolite repair enzyme is conserved in flies.

779F **Role of a phosphoglycolate phosphatase ortholog in responding to hyperosmotic stress in *D. melanogaster***
Salome Ambokadze[1], Jennifer Kennell[21]Vassar College, [2]Biology, Vassar College

Osmoregulation is the active process of maintaining the fluid balance and concentration of electrolytes, carried out by osmoreceptors that detect alterations in osmotic pressure of an organism. One enzyme of interest in regulating hyperosmotic stress is Phosphoglycolate Phosphatase (PGP). It belongs to a class of well-conserved haloacid dehalogenase (HAD)-like hydrolases that catalyze the de-phosphorylation of non-protein substrates, including small metabolites. In mammals PGP is involved in the cleanup of toxic by-products of glycolysis, serving as a metabolite repair enzyme. Relevant to osmoregulation, PGP has also been shown to act upon Glycerol-3-phosphate (Gro3P) in mammals and worms to form glycerol. While known to be crucial in primarily metabolic pathways, glycerol plays an essential role in protecting many organisms against high salinity environments. *C. elegans* has three predicted *PGP* orthologs (*pgph-1,2,3)* and worms mutant in all three are more sensitive to excess NaCl, due to an inability to synthesize glycerol. Based on sequence similarity, the ortholog of the *PGP* gene in *D. melanogaster* is *CG5567*, although its paralogs (*CG5577, CG32488, CG32487)* could also be performing functions closely related to that of PGP. Interestingly, Fly Cell Atlas scRNA-seq for *CG5567* shows high expression in malphigian tubules, the slender branching tubules extending from the GI tract that absorb solutes, water, and waste, equivalent to a vertebrate kidney and liver. We found that, similar to *C. elegans,* a null mutation in the predicted *PGP* ortholog in flies, *CG5567,* leads to hypersensitivity of adults to high salt stress. Similarly, ubiquitous knock down of *CG5567* via RNAi sensitizes adult flies to high salt stress as does knock-down specifically in the malpighian tubules. We are currently looking at whether the expression of *CG5567* increases in response to salt stress and if this hypersensitivity in *CG5567* mutants is due to decreased glycerol levels in the malpighian tubules. Together our data provide further support that *CG5567* encodes an ortholog of Phosphoglycolate Phosphatase in flies and that its role in regulating hyperosmotic stress response is highly conserved.

780F **Ring neurons in the *Drosophila* central complex comprise a rheostat for sensory modulation of aging**
Christi M Gendron[1], Tuhin S Chakraborty[2], Cathryn Duran[2], Thomas Dono[2], Scott Pletcher[21]Molecular and Integrative Physiology, University of Michigan, [2]University of Michigan

Sensory perception modulates aging, yet we know little about how. An understanding of the neuronal mechanisms through which animals orchestrate biological responses to relevant sensory inputs would provide insight into the control systems that may be important for modulating aging. Here, we provide new insight into how the perception of dead conspecifics, or death perception, which elicits behavioral and physiological effects in many different species, modulates aging in the fruit fly, *Drosophila melanogaster*. Co-housing *Drosophila* with dead conspecifics decreases fat stores, reduces starvation resistance, and accelerates aging in a manner that requires both sight and the serotonin receptor 5-HT2A. We demonstrate that a discrete, 5-HT2A-expressing neural population in the ellipsoid body of the *Drosophila* central complex, identified as R2/R4 neurons, acts as a rheostat and plays an important role in transducing sensory information about the presence of dead individuals to modulate aging. Expression of the insulin-responsive transcription factor foxo in R2/R4 neurons and insulin-like peptides dilp3 and dilp5, but not dilp2, are required, with the latter likely altered in median neurosecretory cells after R2/R4 neuronal activation. These data present an important step forward

toward unraveling the neural underpinnings of how perceptive events impact physiology across taxa.

781F **Genotype-Sex-Device Interactions Impact Differential Expression of Exercise-Related Genes and Phenotypes in Wild-Derived *Drosophila*** Tolulope R Kolapo[1], Annie Backlund[1], Miled A Maisonet Nieves[2], Jordan Albrecht[3], Mckenzie Chamberlain[3], Alyssa Koehler[4], Megan Lawson[4], Abigail Myers[4], Laura Reed[31]Biological Science, University of Alabama, [2]Biological Science, University of Puerto Rico, [3]Biological Sciences, University of Alabama, [4]University of Alabama

Obesity is an epidemic disease that predisposes people to other metabolic related disorders such as cardio-vascular disease. Factors ranging from genetic variation, nutritional imbalance, and lack of physical activity contribute to obesity. Treatments suggested for obesity have emphasized lifestyle modification as a sustainable and efficient solution to obesity related disorders. Of the lifestyle modifications, regular exercise is critical to improve human health and restore cardiac functions.

Studies of genotype-by-exercise effects in human face the challenge of excessive cost, small sample size and other uncontrollable factors. However, *Drosophila melanogaster* is a robust model organism for studying human related disorders because of its shared homology, conserved genetic pathways with humans and short generation time. The innate negative geotaxis behavior of flies has been utilized to develop the TreadWheel and Power Tower as two exercise devices. The Power Tower has a repeated drop and hit mechanism where flies are forced down their vials repeatedly. The TreadWheel uses an end-over-end rotation of vials to change the orientation of vials continuously to keep flies moving upward. Improved climbing performance, reduced triglyceride levels, increase protein level, among others reveal that the two devices exercise flies.

However, the gently inducing mechanism of TreadWheel and more forceful method of the Power Tower are distinct operating mechanisms that might trigger different phenotypic responses. Our study seeks to identify the differences and the effect of genotypes in flies' response post training on both devices. We studied seven wild-derived strain of the *Drosophila* Genetic Reference Panel for their climbing performance, four of these strains were randomly selected to be analyzed for aging and metabolic trait assays. We also conducted gene expression analysis of three DGRP lines to detect differential upregulation of exercise-related genes on both devices.

Our results revealed a significant effect of device on the climbing performance of flies. We also report a significant interaction effect of genotype, sex, and device in differential expression of exercise-related genes at $P<0.001$. In conclusion, flies trained on TreadWheel and Power Tower show distinct responses in some of the measured trait, with genetic variation and sex as major contributors to the training response.

782F **Metabolic requirements during *Drosophila* oogenesis** Emily Wessel[1,2], Daniela Drummond-Barbosa[1,21]University of Wisconsin- Madison, [2]Morgridge Institute for Research

Stem cell lineages can undergo drastic morphological, functional, and metabolic shifts throughout differentiation. For example, the *Drosophila melanogaster* ovary is supported by germline stem cells whose progeny undergo extensive differentiation involving many nutritionally regulated processes to produce mature eggs. Previous work from our lab showed that diet and diet-dependent signaling control *Drosophila* oogenesis at multiple steps. However, it remains unclear how the physiological regulation of the ovary is integrated with germ cell metabolism. We are currently investigating metabolic requirements during *Drosophila* oogenesis in the context of dietary regulation.

783F **Lamp1, lipid transport, and Parkinson** Satya Surabhi[1], Norin Chaudhry[2], Zohra Rahmani[3], Gustavo MacIntosh[4], Serge Birman[3], Andreas Jenny[11]Developmental and Molecular Biology, Albert Einstein College of Medicine, [2]Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, [3]Genes Circuits Rhythms and Neuropathology, Brain Plasticity Unit, ESPCI Paris, [4]Biochemistry, Biophysics and Molecular Biology, Iowa State University

Aging-associated diseases are an increasing socio-economic burden despite efforts to improve healthspan. Pathologies that cause degeneration of the nervous system are particularly devastating, and in many cases are associated with lysosomal malfunction and a decline in proteostasis. Prime examples are Parkinson and Alzheimer's disease that are characterized by accumulation of insoluble protein aggregates that lead to neuronal decay. Lysosomal degradation of cytoplasmic components depends on proper transport of hydrolases from the Golgi to lysosomes where they encounter the acidic environment necessary for their activation. Lysosomes are also important regulatory hubs that integrate nutritional signals and participate in lipid metabolism, and these diseases are associated with alterations in lysosomal pH

and accumulation of lipids, particularly cholesterol in the lysosome.

Drosophila Lamp1 is a bona fide homolog of the mammalian LAMP1/2 proteins that have with partially redundant roles in autophagy and cholesterol assimilation. Consistently, we find that *Drosophila* Lamp1 localizes to late endosomes and lysosomes. However, surprisingly and in contrast to *Lamp1/2* in mice, Lamp1 is not required for development, autophagy, or viability, although males have a reduced mean lifespan. On the other hand, *Lamp1* mutant larvae have elevated levels of sterols and diacylglycerols, indicating functions of Lamp1 in lipid transport beyond sterols.

Most interestingly, *Lamp1* deficiency results in an increase in the number of acidic organelles in the fat body, thus identifying a novel role for Lamp1 the regulation of the pH of the endolysosomal system. The connection between Lamp1 function, endolysosomal acidification, and lipid metabolism suggests that Lamp1 function may be connected to neurological disorders. Indeed, we find that mutation of *Lamp1* in adult flies enhances the locomotor defects induced by the expression of α-synuclein^{A30P}, and we thus have identified a novel role for Lamp1 in protecting flies from neurotoxicity in an established Parkinson model.

784F **Phosphatidylcholine can regulate complex I assembly independent of its role in maintaining mitochondrial membrane integrity** Sanjay Saini, Anjaneyulu Murari, Shauna-Kay Rhooms Rhooms, Divya Vimal, Kaniz Hossain, Maximino Villanueva, Edward Owusu-AnsahPhysiology and Cellular Biophysics, Columbia University Irving Medical Center

Mitochondrial complex I (CI) consists of a matrix and membrane domain oriented almost orthogonally to each other. Several phospholipid molecules are intertwined with CI subunits in the membrane domain; however, their function is unclear. Here, we report that phosphatidylcholine (PC) can regulate CI assembly independent of its role in maintaining mitochondrial membrane integrity in Drosophila melanogaster flight muscles. When the intramitochondrial PC transporter, STARD7, is severely disrupted, assembly of the oxidative phosphorylation (OXPHOS) system is impaired and biogenesis of several CI subcomplexes is hampered. However, intriguingly, a restrained knockdown of STARD7 specifically impairs the incorporation of NDUFS5 and NDUFA1 into the proximal part of the membrane domain of CI without affecting the incorporation of CI subunits localized to the distal part of the membrane domain, OXPHOS complexes already assembled, or mitochondrial cristae integrity. Thus, PC can regulate CI biogenesis independent of its role in maintaining inner mitochondrial membrane integrity.

785F **The Role of the Circadian Transcriptome in Aging Photoreceptors** Sarah E McGovern[1], Gaoya Meng[2], Vikki Weake[21]Purdue University, [2]Biochemistry, Purdue University

Advanced age is one of the greatest risk factors for developing eye disease. With an ever-growing elderly population, it is more important than ever to understand the mechanisms underlying ocular dysfunction with age. Our lab studies *D. melanogaster* photoreceptor neurons to comprehensively profile the molecular basis for age-dependent retinal degeneration. Recent ATAC-seq data generated in our lab over the course of aging in photoreceptors identified changes in the transcription factor activity of the core circadian clock components Clock (CLK) and Cycle (CYC). Expression of a dominant negative version of Clock (CLK^{DN}) resulted in widespread changes in gene expression, chromatin accessibility, and light-dependent retinal degeneration. Since we observed extensive changes after CLK^{DN} expression in photoreceptors, we hypothesized that rhythmic gene expression is a critical component of the photoreceptor transcriptome. To profile the photoreceptor rhythmic transcriptome, we collected flies 10 days post-eclosion and 50 days post-eclosion, and performed nuclear RNA-seq of samples collected every 4 hours in 12-hours light, 12-hours dark conditions. At least half of all transcripts exhibited some degree of rhythmic expression. More than 30% of all transcripts had a detectable change in the rhythmic expression pattern with aging, including a subset of transcripts that gained rhythmicity of expression. The histone H3 lysine 4 trimethylation (H3K4me3) mark is associated with active chromatin and decreases genome-wide during aging in photoreceptors. We knocked down the histone methyltransferase responsible for depositing H3K4me3, Set1, in the photoreceptors of young flies and performed nuclear RNA-seq. Strikingly, many changes in rhythmic transcript levels observed in aging were recapitulated in photoreceptors with a Set1 knockdown. Our current work continues to examine the intersection between the circadian rhythm and epigenetics by leveraging multi-omics approaches including CUT&RUN of CLK and CYC to understand the processes underlying age-dependent retinal degeneration.

786F **Exercise Mimetics As A Rescue For Mobility Phenotypes In a *Drosophila Clock* mutant** Maryam Safdar, Robert WessellsPhysiology, Wayne State University School of Medicine

Disturbances in circadian rhythms are associated with various negative health outcomes, which include an increasing incidence of chronic diseases with high societal costs. While endurance exercise can positively impact the downstream negative effects of rhythm disruption, it is not available to all patients impacted by sleep disruptions, in part because sleep disruption itself reduces exercise capacity. Therefore, there is a need for therapeutics that bring the benefits of exercise to this population. One method to discover these therapeutics is to investigate the relationship between endurance exercise and circadian rhythm disturbances using a *Drosophila* model of rhythm loss, the Clk^{out} mutants. These mutants have been well established to have a disrupted circadian rhythm of activity and sleep due to a lack of clock protein production. Our lab has shown them to also have the phenotypes of reduced exercise capacity, measured as a post-training endurance, flight performance, and climbing speed. Rescue will be accomplished using a genetic method via Sestrin overexpression and a pharmacological rescue via Octopamine feeding. Both molecules, Sestrin and Octopamine, are well-known mediators of exercise and the methods listed above have been shown to mimic exercise in sedentary *Drosophila*. Sestrin overexpression has also, previously, been shown to rescue neurodegenerative mobility phenotypes in *Drosophila*.

787F **Serotonin signaling ties brain and peripheral metabolism to influence aging in *Drosophila*** David Boettger[1], Scott D Pletcher[2], Yang Lyu[31]College of Literature, Science, and the Arts, University of Michigan, [2]Department of Molecular and Integrative Physiology and Geriatrics Center, University of Michigan, [3]Department of Molecular Biology and Biochemistry, Rutgers University

Environmental exposures are profoundly influential on the lifespan and health of animals. Neural mechanisms involved in perceiving the environment have been documented as drivers of the longevity-control pathways. A critically important, but often overlooked aspect of these studies, however is that most of them focus on one environmental variable—yet in reality, animals (including humans) are exposed to multiple, sometimes conflicting, internal and external signals. How the brain integrates multiple environmental cues, and how these neuronal activities are summed to influence aging and age-related diseases remains to be determined.

Decision-making is the key neuronal process that integrates distinct signals to mediate adaptive responses. As the major focus on the decision-making processes has been immediate output (e.g., changes in neuronal activities or behaviors), little attention has been paid to the slower, long-term outcomes of decision-making. We have established a nutrient choice paradigm in *D. melanogaster* to investigate the effects of choice on lifespan and metabolism. We found that when animals are presented with a food environment in which they need to constantly assess and decide between two macronutrients (e.g., carbohydrates and proteins), their behavior, metabolism, and lifespan are affected compared to control animals that are given a single, complete medium. Importantly, we have found that loss of a functional serotonin receptor (5-HT2A) does not affect nutrient preferences, but nearly doubles lifespan in a nutrient choice environment. These effects are independent of consumption and manipulation, indicating that 5-HT2A-dependent circuits are involved in coordinating metabolic and behavioral changes in individual animals in response to physical interactions with their food source. It was unclear how these neuronal activities affect aging and metabolism, but we have very recently discovered that 5-HT2A modulates critical metabolic components in both the brain and the periphery, and we have identified at least two enzymes that are responsible for the choice-induced lifespan alterations. First, we discovered that 5-HT2A influences the activities of the tryptophan-kynurenine metabolism in the brain which affects aging. More specifically, the neuronal expression of *kynurenine formamidase* (*KFase*) is required for the lifespan alterations in animals that are fed on a choice diet. In addition, 5-HT2A mediates a systematic metabolic reprogramming that involves the whole body. Specifically, Glutamate Dehydrogenase (GDH) likely acts downstream of the neural signaling to modulate aging outside of the brain. As GDH is a key component that influences the activities of the TCA cycle, our data suggest that GDH defines the key metabolic aspects in non-neuronal cells to influence energy homeostasis and longevity.

788F **The role of Nemp in nuclear lipid droplets in *Drosophila*** Cole Julick, Bilal A Hakim, Helen McNeillDevelopmental Biology, Washington University in St. Louis

Nemp is a 5 transmembrane inner nuclear membrane protein that we recently discovered is essential for fertility in *Drosophila*, worms, fish and mice (Tsatskis et al., 2020). Recent studies indicate that truncated forms of Nemp lead to lipid droplet (LD) accumulation in *C. elegans* (Mosquera et al., 2021) and loss of Nemp1 leads to increased LDs in mouse oocytes (our unpublished data). LDs are a major form of fat storage and act as an energy reservoir as well as building blocks for membrane growth and repair. LDs are multifunctional organelles that exist in the cytoplasm and in the nucleus-with roles in stress response, cell signaling, and development. While the functions of nuclear lipid droplets (nLDs) are not fully understood, some possibilities include maintaining nuclear lipid homeostasis, storage sites for both normal

and unfolded proteins, hydrophobic proteins, and as detoxication sites of hydrophobic matter. Additionally, nLDs might also be deleterious by disrupting nuclear organization or triggering aggregation of hydrophobic proteins. My research investigates the presence of LDs in both wild-type and *nemp* mutant *Drosophila* and what roles they may be playing in the storage of cell lipids and cell stress. Understanding the role LDs play in the nucleus may be important for the treatment of related human diseases such as lipodystrophies, fatty liver, and obesity.

789F **Anorexigenic protein NUCB1 regulates lipid homeostasis in *Drosophila* by modulating TAG storage** Narsimha Pujari, Marity Dessolain, Adelaine K.W. LeungWestern College of Veterinary Medicine, University of Saskatchewan

Background: Metabolic dysregulation is the leading cause of obesity-related illnesses. Balanced metabolism modulation involves interorgan communication initiated by neuropeptides secreted from the central nervous system (CNS). The anorexigenic and insulinotropic properties of Nucleobindin 1 (NUCB1) have been reported in vertebrate animal models. However, the detailed mechanism of action is yet to be established. NUCB1 is highly conserved between *D. melanogaster* and humans. We hypothesize that dNUCB1 (*Drosophila* NUCB1) regulates energy metabolism in fruit flies via its expression and function in the nervous system.

Methods: The GAL4/UAS system was used to knockdown and overexpress dNUCB1 in the nervous system. Changes in gene expression were determined by qPCR. The overall metabolic consequences of genetic manipulation were analyzed by measuring the mass and feeding behaviour. The excreta quantification method was used for measuring food consumption. The effect on overall lipid storage was estimated by triacylglycerol (TAG) enzymatic assay. A mortality assay with a Kaplan-Meier curve was performed to assess the effect of dNUCB1 genetic manipulation on fly lifespan.

Results and Discussions:

Overexpression of dNUCB1 in the nervous system resulted in an increase in food consumption in young flies. This difference was absent as the flies got older. A significantly higher mass was observed in these flies throughout their lifespans. The increase in mass is often a result of the dysregulation of energy storage. Enzymatic studies revealed a significant increase in triacylglycerol (TAG), the most common form of energy storage across phyla. These physiological changes due to the overexpression of dNUCB1 led to a much higher mortality rate in the flies. These results strongly indicate that dNUCB1 plays a role in energy metabolism by regulating TAG storage.

790F **Metabolic Functions of Glut1 in *Drosophila* Fat and Muscle Tissue.** Louis Betz, Justin DiAngeloPenn State Berks

Obesity reflects imbalances in energy output and storage. The molecules that tissues use to regulate this storage of excess nutrients are not well understood. To identify novel genes important for nutrient storage, a buoyancy screen was performed in *Drosophila* larvae. One gene that was identified in this screen was *Glut1*, a transmembrane protein responsible for glucose uptake in tissues such as the fat body, muscle, neurons, and gut. To study the tissue-specific metabolic functions of this gene, triglycerides, free glucose, and glycogen concentrations were measured in adult flies with either muscle or fat body specific knockdown of *Glut1* using RNA interference. Decreasing *Glut1* in muscle had little effect on lipid and carbohydrate storage. In addition, muscle-specific *Glut1-RNAi* flies climb and fly normally suggesting that *Glut1* knockdown in muscle does not limit muscle function. In contrast to muscle, fat body-specific knockdown of *Glut1* resulted in an overall decrease in triglyceride and glycogen levels in both male and female *Glut1-RNAi* flies. To determine whether this lack of lipid and carbohydrate storage was due to the fat body specific *Glut1* knockdown flies eating less, the overall feeding behavior of these flies was analyzed. Surprisingly, there was no alteration in food consumption indicating that decreasing *Glut1* in fat body cells decreases nutrient storage by limiting metabolic substrates and not by altering feeding behavior. These data suggest that the metabolic pathways that regulate the synthesis or breakdown of triglycerides and glycogen are altered in *Glut1-RNAi* flies. By determining the genes that are affected by a reduction of *Glut1*in the fat body can provide insight into how altering glucose uptake into fat cells alters lipid and carbohydrate storage and ultimately increase our understanding of the molecules necessary to maintain metabolic homeostasis.

791F **The *Drosophila* Estrogen-Related Receptor supports lipid storage in the fat body during larval development** Tess Fasteen, Melody Maniex, Kasun Buddika, Jason TennessenIndiana University

The Drosophila Estrogen-Related Receptor (dERR), the sole ortholog of the mammalian ERR nuclear receptor family, is

necessary for upregulating glycolysis in preparation for larval growth. Previous studies have demonstrated that dERR activates a metabolic switch at the onset of larval development, resulting in the coordinate upregulation of glycolysis, the pentose phosphate pathway, and lactate dehydrogenase expression. When this switch fails in *dERR* mutants, larvae are unable to process adequate amounts of glucose to support growth and die with severe metabolic defects. However, dERR continues to be expressed throughout larval development, raising the possibility that this NR may modulate metabolic gene expression throughout the juvenile growth period. Moreover, earlier studies investigating dERR's activity were conducted in whole organisms, leading us to question how dERR may promote larval metabolism in individual tissues. We are addressing both questions by investigating the role dERR within the larval fat body. Consistent with previous studies in both mice and adult male flies, we find that *dERR* mutants are lean and exhibit decreased triglycerides (TAG) within the larval fat body. Moreover, tissue-specific rescue experiments demonstrate that expression of a *UAS-dERR* construct within only the fat body is sufficient to restore normal larval TAG accumulation. In order to understand the mechanisms that lead to decreased lipid accumulation, we sought to identify transcriptional changes that occur within the fat body of *dERR* mutant larvae. RNA-seq performed on isolated *dERR* mutant fat bodies not only revealed changes in carbohydrate metabolism but also uncovered an unexpected decrease in the expression of genes involved in lipid catabolism such as fatty acid metabolism and peroxisomal lipid metabolism, although dERR is not thought to directly regulate the expression of these genes. To better understand the link between dERR and changes in fat metabolism, we analyzed *dERR* mutants using a quantitative lipidomic approach. The resulting data revealed a decrease in acylcarnitines and increased levels of fatty acids, phosphatidylethanolamine, and several other lipids. Overall, our findings indicate that dERR is required during larval growth to support proper lipid metabolism and hint at a mechanism by which dERR interacts with other conserved regulators of lipid metabolism.

792S **A *Drosophila* model of Paclitaxel-induced sensory hypersensitivity** Sreepradha Sridharan, Michael J GalkoGenetics, MD Anderson Cancer Center

Some chemotherapy agents cause sensory discomfort and pain that persists past the treatment phase. Paclitaxel (PTX), a chemotherapeutic that affects microtubule dynamics, is known to induce thermal nociceptive hypersensitivity and sensory neuron damage in human patients and in Drosophila larvae. We used a modified feeding protocol to establish a Drosophila model of PTX-induced hypersensitivity. Larvae continuously fed PTX exhibited hypersensitivity to mechanical and thermal stimuli. Thermal hypersensitivity is observed at low concentrations of PTX (even below 1 µM per ml of food), begins within 8 hours of PTX feeding, and does not completely resolve at the larval stage. Live imaging of peripheral thermal nociceptors showed that this hypersensitivity precedes observable neuronal damage. We are now using this model of PTX-induced hypersensitivity to explore the molecular/genetic basis of this hypersensitivity. Of particular interest are pathways (TNF, Hedgehog, and Tachykinin) which regulate onset of acute damage-induced hypersensitivity and insulin-like signaling (ILS) which regulates the persistence of damage-induced hypersensitivity. Preliminary data showed that the insulin-like peptide 4 (ILP4), is required for full PTX-induced thermal hypersensitivity. This suggests that the ILS pathway may regulate PTX-induced thermal nociceptive hypersensitivity and we are currently exploring this pathway further. In summary we have developed a new model of chemotherapy-induced hypersensitivity that allows dissection of the interplay between neuronal damage and hypersensitivity and dissection of the molecular basis of the latter. We will report on any new findings since the submission of this abstract and expect this system to identify conserved regulators of PTX-induced hypersensitivity.

793S **Sugar-free flies: Decreasing Glut1 in all *Drosophila* neurons blunts lipid and carbohydrate storage.** Matthew Kauffman, Justin DiAngeloPenn 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. *Glut1* is a gene encoding 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 aim to characterize the role of *Glut1* specifically in neurons. To do this, we decreased *Glut1* levels by inducing RNAi in all neurons and measured the levels of glycogen, free glucose, and TAG in adult flies. The storage of glycogen, free glucose, and TAG was decreased in *Glut1-RNAi* males; only a decrease in glycogen was observed in the *Glut1-RNAi* female flies. To determine if this decreased storage of glycogen and TAG was due to less food consumption, capillary feeding (CAFÉ) assays were performed. Interestingly, neuron specific *Glut1-RNAi* flies ate similar amounts as controls indicating that decreasing glucose uptake into neurons doesn't alter fly feeding behavior. Previous studies have shown that inducing *Glut1-RNAi* specifically in the insulin-producing neurons in the fly brain decreased insulin-like peptide secretion, perhaps providing a mechanism for the decreased nutrient storage

observed here when *Glut1* is decreased in all neurons. Together, these data reveal that glucose uptake into neurons via Glut1 serves as a sensor to nonautonomously regulate carbohydrate and lipid storage in the fly to control metabolic homeostasis.

794S **Highly conserved shifts in ubiquitin-proteasome system (UPS) activity promote mitochondrial health during aging** Parul Gupta, Sibiao Yue, Lei Wang, George deMartino, Matthew SieberUniversity of Texas Southwestern

Defects in cellular proteostasis and mitochondrial function drive many aspects of infertility, cancer, and other age-related diseases. Moreover, lifespan is extended in long-lived organisms by restoring mitochondrial function and cellular proteostasis. However, despite the importance of these processes to normal aging little is known about the direct mechanistic links between mitochondria and proteasome and how these may enhance longevity and health span. Recent work from our lab has elucidated an evolutionarily conserved direct link between proteostasis and mitochondrial function during quiescence. We have found that GSK3 recruits proteasome to mitochondria by phosphorylating mitochondrial proteins such as VDAC and TOMM20. This recruitment drives mitochondrial remodeling, reduces redox stress, and protects quiescent cells for prolonged periods of dormancy. Interestingly, we have found that this proteasome recruitment mechanism is activated in long-lived animals in C.elegans, Drosophila, and mice suggesting proteasome recruitment to the mitochondria may be a highly conserved mechanism that prevents the widespread mitochondrial dysfunction that is commonly observed during aging. We further looked at effect of PI31, an adapter between proteasome and motor proteins, on this process. We found PI31 knockout causes reduced proteasome recruitment to the mitochondria, reduced respiration, and a major remodeling of the mitochondrial proteome. This implies a vital role of PI31 in the mitochondrial recruitment of proteasomes. This work defines a process that coordinates dynamic regulation of UPS activity to coordinated shifts in mitochondrial metabolism in Fungi, Drosophila, and mammals during cellular quiescence and organismal aging. Moreover, these findings suggest that targeting proteasome recruitment to the mitochondria provides a therapeutic platform to extend health span and treat many age-associated diseases such as Alzheimer's disease that are driven by defects in mitochondrial metabolism and proteostasis.

795S **Genotype and sex impact the response to altered activity behaviors** Heidi Johnson, Nicole C RiddleBiology, University of Alabama at Birmingham

Humans impact ecosystems in various ways. For example, they can affect the movement patterns of wild populations. Distance traveled, territory sizes, as well as type and timing of activity all can shift after anthropogenic disturbance. These diverse changes to movement patterns have the potential to drastically alter individual organismal fitness and population health. While altered movement patterns have been observed across multiple species, their consequences are not well understood. Nor it is clear how variable the individual responses are between the sexes and across different genetic backgrounds. We use a Drosophila exercise system to mimic human disturbance, induce altered movement patterns and define impacts. Using males and females from four genotypes of the Drosophila Genetic Reference Panel, we increase animal movement during a period of innate rest for either 5 or 20 consecutive days. We recorded a wide range of health and fitness measures including survival and baseline activity level. Lifespan and activity levels are strongly dependent on sex and genotype. While genotype and sex are significant parameters determining survival, the 5-day disturbance treatment did not significantly alter lifespan or movement patterns beyond the treatment period. The experiments mimicking a long-term, 20-day disturbance event are on-going. These experiments will investigate if extended periods of disturbance impact either survival or body condition. Together, these experiments will reveal how both short- and long-term disturbance impact animals in a model species, leading to testable predictions for additional studies in natural populations.

796S **Identification of CRISPR mutations in the Zwischenferment (Zw) gene in the model organism *Drosophila melanogaster*** Kathleen Miller[1], Morgan Grace[2], Michael Toneff[1], Alexis Nagengast[21]Biology, Widener University, [2]Biochemistry, Widener University

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency in the world. Many individuals who have this deficiency are often unaware until they are in crisis due to consumption of foods that trigger hemolytic anemia. Once in crisis, red blood cells begin breaking down rapidly and immediate medical attention is necessary. To study glucose-6-phosphate dehydrogenase, the homolog gene Zwischenferment (Zw) was examined in the model organism *Drosophila melanogaster*. Zw codes for the conserved G6PD enzyme which is the rate limiting step in the pentose phosphate pathway (PPP) and provides NADPH required for the detoxification of reactive oxygen species (ROS) and lipid biosynthesis under high nutrient conditions. In both humans and *Drosophila*, G6PD is alternatively spliced

in the 5' UTR to produce two different protein products that vary in length and initial N terminal sequence. We targeted a CRISPR mutation in the second exon of Zw that would disrupt the longer isoform and allow for the production of only the shorter isoform. Genomics preps followed by PCR and sequencing are being conducted to identify potential CRISPR mutations. We discovered a potential CRISPR mutation resulting in a two nucleotide base deletion which would cause a frame shift mutation. In the future, we will continue to examine fly lines in hopes of finding more potential CRISPR mutations that affect the Zw gene function of the different isoforms related to ROS detoxification and lipid biosynthesis.

797S **PrecisionTox – Using *Drosophila* to Redefine Chemical Safety Testing** Shannon Smoot[1], Jessica Holsopple[1], Emma Rose Gallant[1], Ellen Popodi[1], Brian Oliver[2], Thom Kaufman[1], Jason Tennessen[11]Indiana University, [2]NIH

Human industries generate over 350,000 chemicals, however, many of these compounds have not been adequately studied for environmental safety or effects on human health. To address this pressing, global issue, the European Union recently funded a pilot study by the PrecisionTox consortium, with the goal of establishing a high-throughput chemical safety screening pipeline. This international consortium, which consists of labs from 14 universities, is tasked with using six model organisms/systems (*C. elegans, Daphnia, Drosophila,* zebrafish*, Xenopus,* and human cell lines) to determine how 250 candidate compounds disrupt gene expression and metabolic flux. The Tennessen lab, in collaboration with Brian Oliver's Lab at NIH and the Bloomington *Drosophila* Stock Center, are responsible for conducting the *Drosophila* chemical exposures. Towards this goal, we have established a high-throughput exposure protocol for generating dose-response curves and collecting samples for RNAseq and untargeted metabolomics. Our initial studies are focused on an initial library of 50 compounds. Many of these compounds have never been studied in *Drosophila* and our preliminary studies reveal several unexpected phenotypes. Notably, we find that many chemicals produce dramatic sex-specific differences in terms of lethality and feeding behavior. In collaboration with Brian Oliver, we are now investigating the molecular mechanisms that underlie these sex-specific differences.

798S **Regulation of Apolipoprotein-E lipid dysmetabolism linked with Alzheimer's Disease** Ruan Carlos Macedo de Moraes[1], Jonathan Roth[1], Christopher Livelo[1], Hailey Mao[1], Stephen Barnes[2], Girish C. Melkani[11]Department of Pathology, The University of Alabama at Birmingham, [2]Department of Pharmacology and Toxicology, The University of Alabama at Birmingham

The largest genetic risk factor in Alzheimer's Disease (AD) is the presence of the ε4 allele of apolipoprotein E (ApoE). Apolipoproteins are proteins that bind lipids and are responsible for lipid transport and storage. Despite decades since the discovery of the relationship between ApoE ε4 and AD, the role of ApoE ε4 in the pathology of AD is not defined. Recent studies have shown that there is a relationship between the presence of ApoE ε4 and circadian rhythm disruption (CRD) in AD patients. Therefore, our group intends to use an innovative approach using *Drosophila melanogaster* as a model to study the relationships between different ApoE alleles and brain cell metabolism, and the effects of this interaction on sleep and wake cycles. Using *Drosophila* models of AD, we will test the hypothesis that aging, CRD, and phospho-Tau increase the risk for AD by promoting ApoE-induced dyslipidemia and that interventions to support healthy circadian rhythms delay AD pathogenesis. We found that *Drosophila* with different ApoE alleles (ε2, ε3 and ε4) expressed in neurons had accumulation of lipids in the brain, with apoE ε2 resulting in a higher accumulation than apoE ε4. In contrast, when expressing ApoE in another tissue, such as muscle, no enhanced accumulation of lipids was observed, suggesting this is a specific characteristic of brain cells. Using computational tools and an RNAseq database analysis we identified that a selected group of lipid metabolism-related proteins (including DGAT2) are differentially expressed in the hippocampus of ε4 carriers compared with non-ε4 carriers. We found that brain-specific expression of Apoe ε4 has decreased DGAT2 expression, an enzyme that catalyzes the synthesis of triglycerides, compared with ε3 in young flies, though this effect was demolished in the old flies' brains. Our preliminary lipidomic analysis of flies' brains after expression of ApoE ε4 identified a significant decrease in the amount of specific triglycerides compared to age-matched control and an increase in the amount of phosphatidyl ethanolamines (PE) and phosphatidylcholines (PC) after ApoE ε4 expression. Our results demonstrate a possible relationship between the presence of ApoE ε4 and metabolic disruptions in the context of AD.

799S **Developmental Exposure to PFOA alters Lipid and Carbon Metabolism in *Drosophila melanogaster*** Eric A. Kilbourn, Jason M. TennessenBiology, Indiana University Bloomington

Per- and Polyfluoroalkyl substances (PFAS) are highly stable pollutants that poses a global risk to public health. Surveys of the general population have found that the majority of the United States population have measurable levels of these compounds within their blood. One of the most prevalent PFAS molecules is PFOA, which contaminates the environment

of all seven continents and pollutes the water supplies of over 100 million U.S. residents. Considering that the estimated soil half-life of PFOA exceeds 1,000 years, humans will be potentially exposed PFAS contaminants for generations. Moreover, PFOA is readily absorbed by our digestive system, sequestered within internal organs, and is resistant to both metabolic degradation and excretion. Despite the serious health concerns surround PFOA and related molecules, the mechanisms by which these pollutants disrupt animal physiology and metabolism are strikingly understudied. To address this shortcoming, we use *Drosophila* as a model to understand how PFOA alters metabolic homeostasis. Our preliminary results reveal that larvae fed PFOA display abnormal lipid droplet accumulation within the fat body. Moreover, quantitative lipidomics analysis reveals that PFOA exposure is correlated with significant changes in free fatty acids and other lipid species. Finally, targeted GC-MS metabolomics studies reveal that PFOA disrupts carbohydrate metabolism. Overall, our studies reveal that PFOA induces similar metabolic phenotypes in both flies and mammals, thus establishing *Drosophila* as a promising model for the investigating a dangerous class of chemicals.

800S **The impact of time-restricted feeding on cardiac function under metabolic challenges** Yiming Livelo[1], Hiep Le[2], Jordan Rutledge[1], Satchidananda Panda[2], Girish Melkani[11]University of Alabama at Brimingham, [2]Salk Institute for Biological Studies

Cardiovascular diseases (CVDs) have been the leading cause of death, accounting for approximately one in every four deaths in the United States. The leading risk factors of CVDs are age, obesogenic challenges, shift work, and aberrant eating/sleeping patterns. A dietary intervention known as time-restricted feeding (TRF) in which all calories are consumed ≤ 12 hours during the active phase, has been shown to have overall beneficial effects on multiple tissues including the liver, muscle, and heart. Specifically, we previously applied temporal gene expression profiling and genetic validation that led to the discovery of TRiC chaperonin and ETC as pathways mediating TRF benefits in the heart (Gill et al, *Science* 2015). To assess whether TRF can sustain cardiac health under metabolic challenges including obesity, and circadian disruption, we explored the effect of TRF (food access limited to daytime 10 hours every day) on cardiovascular physiology and cytology under multiple metabolic challenges in *Drosophila melanogaster*. We demonstrated that TRF ameliorates the adverse effects of aging, obesity, and circadian disruption on cardiac function and structure. Moreover, a period of 2-week TRF treatment in young flies sustains cardiac function in old age. The future direction will be focused on the common and distinct mechanisms of TRF-mediated benefits on cardiac health under different metabolic challenges.

801S **Investigating the molecular mechanisms driving lipid metabolic changes induced by intermittent, time-restricted feeding (iTRF)** Jared A Gatto, Matthew Ulgherait, Ma Long, Jordana Barnett, Samantha Tener, Carly Y Lam, Andres E Martinez-Muniz, Mimi Shirasu-HizaGenetics and Development, Columbia University Medical Center

Today, we live in an age of unprecedented access to food. Recent research suggests that many Americans eat from the time they wake up to the time they go to sleep. Night eating, specifically, is linked to several aging-related comorbidities, including obesity, cardiovascular disease, and type-2 diabetes. While much ongoing research investigates the mechanisms by which dietary components affect metabolism, it is less understood how the timing of feeding affects metabolism. To this end, dietary interventions that alter the timing of feeding have been shown to protect many aspects of health, even without reducing caloric intake. Time-restricted feeding(TRF) diets have been shown in mice and humans to reduce oxidative stress and inflammation, decrease insulin resistance, lower blood sugar. In mice, TRF has been shown to reduce fat levels, protect against a high-fat diet, and prevent obesity. Using Drosophila melanogaster, our lab developed a robust TRF diet that extends lifespan and delays molecular signs of aging, such as protein aggregation, and showed that TRF enhances circadian gene expression and requires the circadian clock to confer lifespan benefits. In addition, we found that TRF seems to reprogram lipid metabolism; after TRF treatment, flies responded to fasting by utilizing lipids much faster than controls, leading to increased rate of triacylglyceride loss and starvation sensitivity. I found that this TRF-accelerated lipid usage, like TRF-induced lifespan extension, requires circadian components but, unlike TRF-induced lifespan extension, does not require autophagy components. Because the underlying mechanisms remain unclear, I propose to identify molecular components that drive the effects of TRF on lipid metabolism. I will use Drosophila melanogaster, an advantageous model organism for this work because many mammalian metabolic pathways are conserved in flies; flies have short generation time (2-3 months) and flies offer a plethora of powerful genetic tools. These experiments will determine the molecular mechanisms connecting TRF to lipid metabolism and how TRF can beused to ameliorate obesity-and aging-related pathologies. This will improve our understanding on how TRF canconfer health span benefits while alsotestingits therapeutic potential inaging-related,diet-induced obesity.

802S **Actβ is required to prevent purine catabolism and maintain glycogen homeostasis** Heidi S Bretscher, Michael O'ConnorGenetics, Cell Biology and Development, University of Minnesota

In order to ensure enough energy is available to carry out basic life functions, energetically expensive processes must be tightly regulated. Lack of regulation can lead to unnecessary synthesis and breakdown of molecules depleting energy that could be stored for future use or used to carry out basic life functions. Breakdown and synthesis of molecules is controlled at the organismal level by a wide range of signaling pathways. We have uncovered a new role for the TGFβ/Activin ligand Actβ in maintaining energy homeostasis. Actβ positively regulates glycogen (which is synthesized from glucose) storage in both the muscle and fat body. Thus *actβ* mutants have a significant reduction in glycogen, suggesting that Actβ is necessary to prevent glucose from being consumed by other metabolic processes. We find that *actβ* mutants have increased mitochondrial membrane potential, indicative of increased oxidative phosphorylation, yet no corresponding increase in ATP. Using a metabolomics approach, we fine that *actβ* mutants show increased purine nucleotide catabolism. In support of this, we see increase hydrogen peroxide (which is generated during purine catabolism) in the tubules as well as increased peroxisome number in the skeletal muscle. Furthermore, while *actβ* mutants produce only rare adult escapers with severely decreased locomotion, suppressing purine catabolism greatly increases the number of viable adults and improves locomotion. Therefore, we propose that Actβ is necessary to prevent purine nucleotide catabolism, which consumes unnecessary glucose and reduces adult viability and locomotion. Interestingly, this is similar to what is seen in humans with Glycogen storage disease V/McArdle's disease, which is caused by a skeletal muscle mutation preventing glycogen mobilization. Patients with McArdle's disease suffer from muscle fatigue during exercise resulting in increased purine catabolism.

803S **The effects of circadian disruption on behavior, lifespan, and fecundity in *Drosophila melanogaster*** Isaiah Thomas, Elyse Bolterstein, Aaron SchirmerBiology, Northeastern Illinois University

Circadian disruptions can have adverse behavioral and/or physiological effects on organisms impacting their overall health and reproductive fitness. We disrupted the circadian biology of *Drosophila melanogaster* through exposure to excessive nighttime light. We used locomotor, lifespan, and fertility assays to quantify negative effects in Ore-R and W1118 wildtype flies. Control flies were kept in a 12:12 hr light-dark cycle (LD) while the experimental flies were kept in a 12:12 hr light-low-light cycle (LLC) of 4 lux to mimic exposure to artificial-light-at-night (ALAN), a form of photopollution. Our data showed significant behavioral differences in the active-rest phases of wildtype flies, with the LLC group having a longer duration of midday inactivity and reduced lifespan compared to controls. In addition, there were significant sex differences in both locomotion and lifespan as well as preliminary data indicating that LLC conditions affect fecundity. To date, our research confirms that ALAN can have a significant impact on the behavioral patterns and lifespans of *D. melanogaster*, establishing a more convenient model to explore future questions related to circadian disruption.

804S **Male and female adult body size is affected by small differences in vial density** Morgan Magee[1,1], Rebecca Spokony[21]Macaulay Honors College, CUNY, [2]Natural Sciences, Baruch College, CUNY

Many genetic pathways and environmental factors have been shown to affect adult body size. In many *Drosophila* labs, the same fly food vials are used for short term experimental conditions and long term stock maintenance. The standard amount of food can be used to feed hundreds of flies. Popular conditions for genetic crosses utilize 8 females per vial, with an overnight egg lay. Drosophila females lay approximately 50 eggs each day, meaning the average vial would contain 400 larvae, but vial density varies considerably. Since the vials can accommodate so many individuals, we hypothesized that small changes in vial density would not affect adult size. We collected OreR eggs on apple juice plates in four different cages. For each plate, we placed 1, 25, 50, 75 or 100 first instar larvae into vials. Larvae grew at 25 deg C. We then measured pupal length. We found that the females are on average 0.22 mm longer than males at each density ($p<.0001$). Female and male mean pupal length decreased with each increase in density. Both adult male and female size was affected by a difference of 50 larvae ($p<0.01$). The mean size differences between vials with 1 larvae vs 100 larvae is 0.1 mm (f) and 0.12 mm (m). These results indicate that variation in vial density and sex needs to be taken into account when studying Drosophila size.

805S **The ellipsoid body modulates aging in Drosophila** Evelynn Henry[1], Tuhin S Chakraborty[2], Yang Lyu[3], Scott D Pletcher[21]Cellular & Molecular Biology, University of Michigan, [2]Molecular & Integrative Physiology, University of Michigan, [3]Molecular Biology & Biochemistry, Rutgers University

The perception and response to environmental conditions and internal states modulates aging in diverse organisms, including *Drosophila* and *C. elegans*. In some cases, such as nutrient or mate perception, we know a little about the mechanisms involved. Gustatory perception of female pheromones in *Drosophila*, for example, accelerates aging in male flies through circuits involving receptor ppk23 and NPF signaling. In all cases, however, we lack a detailed understanding

of the neural circuits that orchestrate these changes. Our lab has discovered that the ellipsoid body is an important mediator of aging under a variety of conditions, and our data indicate that R2 and R4d ring neurons, specifically, are particularly influential in modulating lifespan. For example, R4d activity promotes survival under nutrient stress and drives changes in behavior and physiology associated with different levels of dietary amino acids. Silencing R2 and R4d extends lifespan in flies exposed to dead conspecifics and in flies fed a "choice diet" in which macronutrients are presented separately. Knocking down the conserved 5-HT_{2A} receptor in R4d neurons recapitulates this lifespan extension, indicating that serotonergic inputs may serve as an evolutionarily conserved system that transduces environmental information to aging outputs. The EB and broader central complex are known to influence a wide variety of phenotypes, but to our knowledge, a role in aging has not yet been reported. Given the known functional analogies between the CX and mammalian brain regions like the basal ganglia, this work may ultimately inform the study of neural control of aging in mammals.

806S **Role of sleep in *Drosophila* brain lipid homeostasis** Elana Pyfrom[1], Paula Haynes[2], Yongjun Li[3], Vishal Kanigicherla[3], Greg Artiushin[4,5], Jessica Schwarz[4], Amita Sehgal[4,61]Genetics, University of Pennsylvania, [2]Pharmacology, University of Pennsylvania, [3]School of Arts and Sciences, University of Pennsylvania, [4]Neuroscience, University of Pennsylvania, [5]Johns Hopkins University, [6]Howard Hughes Medical Institute

Sleep is required for optimal brain function, but how it promotes neuronal health and activity is not well understood. Our recent data using a *Drosophila* model implicates sleep in metabolic homeostasis, particularly in the regulation of lipid metabolism. For instance, we found that acylcarnitines accumulate in *Drosophila* heads under multiple conditions of sleepiness. We recently found that daily sleep-wake cycles regulated the transfer of lipid from neurons to glia during wake and regulated glial lipid droplet accumulation during sleep. Transferred lipids are likely metabolized in glia through beta-oxidation, a process that does not occur in neurons. While the source of these lipids is unclear, the endoplasmic reticulum (ER) is a likely processing and packaging depot, since it promotes lipid droplet formation, phospholipid production, and lipid export to other organelles and cells. Preliminary data showed that sleep amount was reduced by neuronal knockdown of molecules that mediate lipid processing at the ER membrane, further supporting a link between sleep and lipid homeostasis.

807S **Olfaction mediated GABA restricts pyruvate metabolism to regulate blood-progenitor redox homeostasis and development** Manisha Goyal[1,2], Bruce R Cooper[3], Ramaswamy Subramanian[2], Tina Mukherjee[41]Regulation of Cell Fate, Institute For Stem Cell Science and Regenerative Medicine, [2]Department of Biological Sciences, Purdue University, [3]Purdue University, [4]Institute For Stem Cell Science and Regenerative Medicine

Various signalling cues and metabolic pathways are shown to regulate myeloid progenitor development. But, the developmental complexity and diverse immune cell types in vertebrate model systems have restricted a thorough metabolic analysis. As a result, the distinct metabolic process that regulate myeloid cell development and differentiation remains unexplored. *Drosophila* larval lymph gland presents an excellent genetically tractable model system to shed light on these complex issues. We explored how metabolic intermediates are altered in lymph gland blood-progenitor cells and their contribution in maintaining blood-progenitor homeostasis. Our metabolic analysis revealed changes in pathways involved in amino acid, TCA cycle metabolites, and ROS homeostasis. We show that *Drosophila* larval blood-progenitor cells rely on olfaction derived GABA catabolism to regulate ROS homeostasis and control lymph gland growth. This regulation is also important for maintaining progenitor identity and a successful immune-response. GABA catabolism restricts pyruvate entry into TCA cycle by regulating phosphorylation of the inhibitory enzyme pyruvate dehydrogenase kinase (PDK) and controls blood-progenitor ROS homeostasis. GABA catabolism regulates pyruvate shuttling not only into TCA cycle, but it also controls pyruvate fuelling towards gluconeogenesis pathway. Our data shows that GABA catabolism controls glutathione synthesis to regulate ROS homeostasis. Glutathione, a major ROS scavenger is synthesised from cysteine, glutamate and glycine in cells. Utilising isotopic labelling of $U^{13}C$-Pyruvate in lymph glands, we found that blood-progenitor cells incorporate 13C label in serine along with label incorporation in TCA metabolites, showing pyruvate conversion to serine in blood-progenitors. Serine, a component of transsulfuration pathway, contributes to cysteine formation and thus, glutathione synthesis. Overall, we show that pyruvate metabolism is restricted by GABA catabolism, and loss of GABA transporter and its breakdown leads to impaired ROS homeostasis due to an increase in TCA activity and reduction of glutathione synthesis in the progenitor cells. Collectively, these studies highlight that GABA catabolism is essential for normal hematopoiesis by regulating blood-progenitor redox homeostasis. Since, *Drosophila* blood progenitors are akin to vertebrate myeloid lineage, the relevance of the study remains broadly conserved and needs further exploration.

808S ***Drosophila* macrophages regulate sugar metabolism through the fructose receptor** Nuri Cha, Ferdinand KorantengLife Science, Hanyang University

Food is the primary resource of nutrients for insects and flies use their external sensory organs to detect consumable food. There are various types of chemosensory systems, including olfaction, gustation, and mechanical sensation, which are mediated by chemoreceptors expressed in various organs. In *Drosophila*, the sense of taste is perceived through gustatory receptors in the wing, legs, labellum in adults, or terminal and dorsal organs in larvae, which function similarly to mammalian taste receptors. Organs expressing Gustatory receptors (Grs) are connected to sensory neurons, converting external stimuli into neuronal signals to modify the behavior of animals according to their preference. Here, we found that larval hemocytes are an additional cell type that express various sweet- or bitter-sensing gustatory receptors, in addition to the traditional sensory organs. Gr43a, a well-known fructose-sensing gustatory receptor in adult brain (Miyamoto, Slone et al. 2012), is also detected in larval hemocytes. Moreover, we discovered that Gr43a in larval hemocytes senses the level of fructose in the serum to increase intracellular calcium levels in the hemocyte. Knocking down Gr43a in larval hemocytes does not alter the population of hemocytes in general; however, it does increase the levels of both glucose and fructose levels in the serum and decrease intracellular sugar levels. Furthermore, the increased sugar levels in the serum impact the overall metabolism of the larvae by increasing insulin secretion from the brain insulin producing cells (IPCs) and activating lipid storage in the fat body through active insulin signaling. Overall, these results demonstrate that hemocytes directly contribute to metabolic homeostasis in *Drosophila* larvae by sensing and controlling the levels of fructose and glucose in the hemolymph.

Keywords: hemocytes, metabolism, fructose, gustatory receptor, homeostasis.

809S **Assessing the role of genetic variation on chromatin regulation of lifespan and age-related traits in *Drosophila melanogaster*** Devonique L Brissett[1], Jeff Leips[21]Biological Sciences, University of Maryland Baltimore County, [2]University of Maryland Baltimore County

Histone modifications are a distinctive hallmark of the aging process. The aging epigenome is characterized by dynamic changes in patterns of histone modifications and is associated with age-related diseases such as cancer and neurodegeneration. The prevalence of histone H3 lysine 4 trimethylation (H3K4me3) and H4K16 acetylation changes with age, with the direction of this change varying based on the species and cell type in question. Substantial natural variation in lifespan exists across several species. Genetic differences among individuals account for a considerable amount of lifespan variation. However, there remains a critical need to understand the extent to which variation in lifespan is influenced by variation in chromatin regulation. A previous study in our lab has demonstrated that three genotypes from the Drosophila Genetics Reference Panel differ significantly in lifespan. Therefore, we hypothesize that genetic differences between the genotypes promote age-related changes in patterns of H3K4me3 and H4K16ac abundance, leading to lifespan differences. To test this, we are using three genotypes with differing lifespans to quantify the abundance of H3K4me3 and H4K16ac at young, middle-, and old age in mated male and females. We found that the H3K4me3 and H4K16ac levels increased during aging in both males and females. However, the magnitude of the increase of each mark varied based on genotype and sex. We are further investigating the distribution of H3K4me3 and H4K16ac throughout the genome of genotypes with differing lifespans. These findings could be insightful for generating personalized epigenetic therapeutics to improve healthspan and longevity

810S **Investigation of the mitochondria amino acids compartmentalization** Hiroshi Nishida[1,2], Norbert Perrimon[3], Shingo Kajimura[11]Beth Israel Deaconess Medical Center at Harvard Medical School, [2]Department of Genetics, Harvard Medical School, [3]Genetics, Harvard Medical School

Distinct pools of metabolites and metabolic enzymes at a subcellular level provide another layer of flexibility in metabolite utilization, thereby allowing for robust adaptation to a variety of intrinsic cues and external stress. One of the crucial metabolic compartments is the mitochondria. Mitochondria produce not only ATP but also various metabolites to replenish cellular demands. Mitochondria have a highly impermeable inner membrane that doesn't allow any metabolites to pass across without a transporter; thus, it requires designated transporters for each metabolite, which orchestrates mitochondrial metabolite compartmentalization. Mitochondria contain their genome, hereafter mtDNA, which encodes the part of the OXPHOS complex. All the mtDNA-encoded genes are transcribed and translated within the mitochondria, indicating that mitochondria upatake each amino acid as a building block for protein synthesis. However, the vast majority of mitochondrial amino acid transporters remain uncharacterized. To circumvent this issue, we developed a robust experimental platform that enables systemic characterization of mitochondrial transporters

using Drosophila. We found that inhibition of the mtDNA translation caused severe mitochondrial enlargement in the larval fat body. Using this phenotype as a readout, we conducted an RNAi screening targeting the mitochondria localized transportes and identified uncharacterized genes as mitochondria amino acid transporter candidates. Dysregulated metabolite delivery to the mitochondria lies at the heart of metabolic disorders. Our approach contributes to understanding the molecular regulation of mitochondrial metabolite compartmentalization and also provides a new roadmap for reversing disease phenotypes that stem from defects in such processes.

811S **Autophagy in the larval fat body influences growth of the developing wing** Todd J. Fairbanks, Thomas P. NeufeldDepartment of Genetics, Cell Biology, and Development, University of Minnesota

Eukaryotic cells are highly adaptable to their environment, utilizing signaling pathways to maintain homeostasis. Autophagy is the catabolic breakdown and recycling of cytoplasmic constituents through specialized vesicles within the lysosome. The degradation of these constituents allows for energy production and continued biosynthesis when nutrients are restricted. Whereas cell-autonomous roles of autophagy in maintaining metabolism and survival have been established, potential non-autonomous effects of autophagy on the growth and development of neighboring cells and peripheral tissues are less well understood. In *Drosophila*, the larval fat body is thought to serve as an energy storage organ, in which nutrients are mobilized through autophagy to help support the development of other tissues in times of starvation and during metamorphosis. Here, we test this model by disrupting autophagy specifically in the larval fat body, and measuring the effects on the growth, proliferation and survival of the developing wing. Our data show that depletion of Atg14 or Atg17 in the larval fat body significantly influences these parameters under both fed and starvation conditions, and alters overall body mass. Our results suggest that autophagy plays important non-autonomous roles during normal development and in times of stress.

812S **The transcriptional repressor hairy acts in the larval fat body to inhibit Dilp6 expression and whole-animal growth** W. Kyle McPherson[1], Dalton L Hilovsky[2], Shivani Reddy[1], Michelle Bland[11]Pharmacology, University of Virginia, [2]North Carolina State University

The *Drosophila* larval fat body regulates growth of the whole animal in response to dietary nutrients, hormonal cues, and physiological stressors such as infection. The allocation of resources to growth and nutrient storage and the secretion of endocrine growth factors such as Dilp6 underlie fat body-dependent growth regulation. Our previous work has shown that innate immune signaling in the larval fat body decreases transcript and circulating levels of Dilp6. We screened predicted transcriptional regulators of *Dilp6*, identified using ENCODE consortium data, focusing on the transcriptional repressor hairy. Endogenous expression of *hairy* parallels expression of *juvenile hormone-inducible protein 26*, with fat body transcript levels of both genes peaking at ~100 hours after egg lay in the mid-third instar and declining as animals approach the pupal stage. In contrast, *Dilp6* expression in the third instar fat body parallels expression of the ecdysone target *ecdysone inducible-protein 74EF*, with increasing levels from the mid-third instar to the wandering stage. We find that increased expression of hairy in the larval fat body significantly reduces *Dilp6* transcript levels from the mid-third instar to the white prepupal stage. We previously showed that Toll signaling targets Dilp6 to reduce whole-animal growth. Overexpression of hairy in the larval fat body similarly reduces whole-animal growth. Interestingly, co-expression of Dilp6 and hairy transgenes in larval fat body rescued adult viability relative to flies that over-expressed hairy alone. Dilp6 increased growth to the same extent in males regardless of fat body hairy expression, but forced Dilp6 failed to increase growth in female flies that over-expressed hairy in the larval fat body. We noted that expression of hairy in the larval fat body and salivary glands also prevents expression of the salivary glue protein Sgs3 which is required for proper pupation and is a target of ecdysone signaling. Together, these data show that hairy inhibits the developmentally-regulated production of Sgs3 and Dilp6. Our results raise the questions of which developmental cues positively and negatively regulate hairy in the fat body and by what mechanism(s) hairy regulates Dilp6 expression.

813V **The impact of dietary folic acid supplementation on hypoxia on health outcomes in wild-type *Drosophila melanogaster*** Nafisa M JadavjiBiomedical Sciences, Midwestern University

One-carbon (1C) metabolism is a metabolic network that integrates nutritional signals with biosynthesis, redox homeostasis, and epigenetics. It plays an essential role in the regulation of cell proliferation, stress resistance, and embryonic development. The natural form of the B vitamin, folate, is central in 1C, as well as the synthetic form of the vitamin referred to as folic acid. In the cell, 1C plays an essential role in nucleotide synthesis of purines, removal of uracil from DNA, and methylation, through the metabolism of homocysteine and generation of *S*-adenosylmethionine. Our work has previously shown that dietary folic acid supplementation is beneficial after hypoxia, however, the mechanisms

through which this occurs remain unknown. Little is known about the molecular mechanisms underlying the cellular responses to lack of oxygenation and how to prevent damage once a reduction in O_2 supply has occurred. The aim of our study is to investigate the how folic acid supplementation impacts hypoxia outcome. We used W1118 male and female *Drosophila melanogaster*, they were put on folic acid supplemented diets of 50µM and 100µM as well as control diet with the minimal amount of folic acid. Offspring from these crosses were exposed to 2 hours of 1% oxygen for a period of 2 hours after which flies were returned to normoxic conditions to model reperfusion. Mortality rate was recorded for one week after hypoxia treatment. We observed that 50µM of folic acid reduced the number of dead flies whereas 100uM increased the number of dead flies. Furthermore, we observed changes in climbing behavior of flies 24 hours after hypoxia that were supplemented with 100µM of folic acid. We are currently investigating apoptosis and oxidative stress mechanisms in brain tissue of flies to determine potential mechanisms.

814V **The Impacts of Intestinal Dysfunction on Aging and Disease** Anna Salazar, David Grace, Samantha LeChristopher Newport University

The world population is aging, with the number of people over 65 more than doubling to 1.57 billion by 2050, with 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. We have recently developed a *Drosophila* model of intestinal barrier dysfunction through perturbing an invertebrate tight junction equivalent. We have shown that altering the levels of one junction protein, Snakeskin (Ssk), is both necessary and sufficient to cause and restore several important markers of aging. Ssk knockdown leads to rapid and reversible intestinal barrier dysfunction, altered gut morphology, dysbiosis, inflammation, metabolic dysregulation, and a dramatically reduced lifespan. Additionally, perturbing barrier function in the gut has non-cell-autonomous impacts, including alterations in the brain and muscle. These analyses 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. Current research utilizes cell and molecular biological methodologies to build upon this foundation to address crucial questions at the intersection between microbial dysbiosis, epithelial integrity, inflammation, neurodegeneration, and disease, with the ultimate goal of discovering novel therapies that may enhance barrier function, healthspan, and lifespan.

815V **Dietary sugar and protein differentially regulate the insulin and IGF1 homologs Dilp2 and Dilp6 in *Drosophila*.** Miyuki Suzawa, W. Kyle McPherson, Shivani Reddy, Elizabeth E. Van Gorder, Leila A. Jamali, Cami N. Keliinui, Michelle L. BlandPharmacology, University of Virginia

Insulin-like hormones that are secreted in response to dietary nutrients activate the conserved insulin/IGF1 signaling pathway to promote anabolic metabolism and animal growth. In *Drosophila*, seven insulin-like peptides (Dilps) are known to activate insulin/IGF signaling through a single known insulin receptor. To determine whether Dilps respond uniformly to varying environmental conditions, we specially explored how starvation and distinct dietary nutrients affect circulating levels of Dilp2, secreted by insulin-producing cells in the brain, and Dilp6, secreted by the fat body. We used dual-epitope tagged *Dilp2* and *Dilp6* alleles to measure hemolymph levels of each hormone in male and female larvae. Starvation of mid-third instar larvae on agar for 24 hours led to a 90% reduction in circulating Dilp2 but no change in circulating Dilp6 levels. Next, we asked whether Dilp2 and Dilp6 respond to specific dietary nutrients. We fed larvae sucrose and/or yeast extract in agar using a nutritional geometry approach and measured Dilp2 and Dilp6 by ELISA to associate hormone levels with distinct dietary conditions. Hemolymph Dilp2 was dose dependently recovered from starved levels by feeding larvae yeast extract but not sucrose. Interestingly, elevated and imbalanced ratios of sucrose to yeast led to modest reductions in circulating Dilp2. In contrast, circulating levels of Dilp6 were increased by a sucrose-only diet, but surprisingly, hemolymph Dilp6 was strongly suppressed by yeast in the diet. Additionally, we find that whole-animal glycogen and triglyceride storage levels strongly and positively correlate with dietary sucrose and Dilp6 but not dietary yeast and Dilp2. These data show that Dilp2 and Dilp6 secretion are regulated differently by environmental conditions ranging from infection, as shown previously, to dietary composition. Dilp2 and Dilp6 are regulated in completely opposite ways by distinct nutrients. Our work raises the question of how the single known insulin receptor integrates divergent signals from distinct Dilps to control growth and metabolism.

816V **Neuronal expression of Taxi regulates lifespan through Adar in *Drosophila melanogaster*** Upasana Gupta[1], Vanlalrinchhani Varte[1], Fathima Muhamed Ashraf[2], Upendra Nongthomba[11]Indian Institute of Science, [2]Vellore Institute of Technology

Ageing involves deterioration in physiological processes, such as maintenance of neuronal health, muscle, fat bodies, and gut bacteria play a crucial role in the progression of ageing. Although the major pathways involved in ageing have been fairly studied, there are still new molecular players that would help us understand the cross-talk between such factors. In this study, we show that expression of Taxi, a transcription factor, is required to maintain the lifespan in *Drosophila*. Hypermorphic (*jumper*) and hypomorphic (*taxi*^{1}) alleles of *taxi* show reduced lifespan. We have identified that pan-neuronal over-expression and knockdown of Taxi lead to a stark reduction in the lifespan. In our previous study, we show that Taxi negatively regulates *Adar* (Adenosine deaminase acting on mRNA), an ortholog of the human ADAR2, implicated in neurodegeneration, neurotransmission, and other metabolic pathways, mainly known for its mRNA editing activity. Interestingly, over-expression of *Adar* significantly rescued the reduction in lifespan caused by *taxi* over-expression in neurons. Conversely, the knockdown of *Adar* rescued the defective lifespan caused by *taxi* knockdown in neurons. Moreover, Adar also function as an RNA binding protein and regulates expression of its target genes, which may be editing-independent. We show that enzymatically inactive *Adar* also rescued the reduced lifespan of flies having neuronal *taxi* over-expression background. Our work suggests that besides the editing activity, *Adar* may have editing-independent roles implicated in the regulation of lifespan. Using bioinformatic databases and tools, such as FlyFactorSurvey and MEME Suite, we have predicted a Taxi-binding motif upstream of *Adar.* Overall, we show that neuronal tissue-specific controlled expression of *taxi* and its interacting partner *Adar* is imperative in lifespan regulation. Our study warrants further investigation of targets of *Adar* from our RNA-seq data of *taxi* null to elucidate plausible pathways involved in the regulation of lifespan in *Drosophila*.

817V **Evaluation of the genotoxic potential of *Cucurbita pepo* root extracts treated with the herbicide clomazone using the SMART assay on the wing of *Drosophila melanogaster*.** Marco Antonio Carballo-Ontiveros[1], Daniela Nuñez-Ledezma[2], Olivia Alvarado-Lastre[2], Claudia Mónica Flores-Loyola[2], Mariana Camila Muñoz-Ponce Nava[2], América Nitxin Castañeda-Sortibrán[11]Cellular Biology, Universidad Nacional Autónoma de México, [2]Universidad Nacional Autónoma de México

The herbicide clomazone (2-(2-chlorophenyl) methyl-4,4-dimethyl-3-isoxazolidinone (CAS 81777-89-1), is highly soluble in water (1102 mg/L) and weakly to moderately persistent in soils, with half-lives (t 1/2 s) ranging from 5 to 60 days. It is used to kill broadleaf weeds and some grasses that infest various crops. Its toxicity is moderate in mammals and it acts generally by inhalation and ingestion. Due to its use in a large number of crops, its high solubility in water and its moderate persistence in the soil, it is important to know the genotoxicity of clomazone, which was evaluated through the SMART assay on the wing of *Drosophila melanogaster*. For this purpose, the extracts of roots of *Cucurbita pepo* treated with clomazone in concentrations of 2.5, 5 and 10% were tested in third instar transheterozygous larvae obtained from the standard cross (ST), which do not express basal levels of cytochromes P450, of *flr*^{3}/*In*(3LR)TM3,*Bd*^{5} virgin females with *mwh*/*mwh* males. Distilled water was used as a negative control. Of the adults obtained from the exposed larvae, their wings were collected to mount permanent slides. Reading the preparations made it possible to identify and record the expression of the *flr*^{3} and/or *mwh* marker genes to statistically process the data in the SMART V2.0 software. The results obtained in the present work indicate that the extract of *Cucurbita pepo* roots treated with clomazone is genotoxic in the three concentrations used.

818V **The neurodegeneration gene *iPLA2-VIA* is required for mitochondrial maintenance in the *Drosophila melanogaster* female germline, with autonomous and non-autonomous components** Eliezer Heller[1], Samuel Intrator[1], Sarah Liberow[1], Nicole Soussana[1], Josefa Steinhauer[21]Biology, Yeshiva University, [2]Yeshiva University

Loss of function mutations in the gene *PLA2G6*, encoding the group 6A calcium-independent phospholipase A2, iPLA2-VIA, are associated with severe ND in humans, including autosomal recessive dystonia-parkinsonism. The orthologous *Drosophila melanogaster* gene encodes a highly similar protein, and loss of function mutations in flies lead to neuron loss and age-dependent locomotor defects. We have discovered a novel role for iPLA2-VIA in the *Drosophila* female germline. In wild-type flies, *iPLA2-VIA* is highly expressed in adult germ cells, and a transgenic HA-tagged iPLA2-VIA protein (PB isoform) localizes to the mitochondria of ovarian nurse cells. Furthermore, a null mutation in *iPLA2-VIA*, generated in our lab, causes age-dependent mitochondrial aggregation and loss of mitochondrial membrane potential in the female germline, symptoms of mitochondrial damage, with eventual germ cell apoptosis. The mitochondrial localization of the protein and the cellular effects in the mutant mirror the behavior of this gene in neurons, suggesting possible common underlying mechanisms of action in these two cell types. Because *iPLA2-VIA* is expressed broadly in somatic tissues as well as in germ cells, we explored the tissue autonomy of the germline mitochondrial defects using our method to quantitatively analyze nurse cell mitochondrial aggregation. Surprisingly, germline *iPLA2-VIA* RNAi knockdown only mildly phenocopied the null mutation, while ubiquitous somatic knockdown led to strong mitochondrial aggregation and death

of female germ cells, suggesting a critical non-autonomous component to the germline mitochondrial defects. Further experiments showed that *iPLA2-VIA* knockdown specifically in neurons led to very strong germline mitochondrial defects. Feeding assays indicated that the germline defects produced by neuronal knockdown were not simply a byproduct of starvation in the knockdown flies. We currently are testing whether knockdown in other somatic tissues, including muscles, fat body, or somatic ovarian follicle cells also impacts the germline. Our findings suggest an unanticipated role for neuronal maintenance in germ cell health during aging.

819V **A comparative study of lifestyles and metabolism of *Drosophila lutzii*, a floridosa group of species, and sympatric *D. simulans*, a generalist specie** Juan M. Murillo-Maldonado[1], Juan R. Riesgo-Escovar[21]Universidad Nacional Autónoma de México, [2]Universidad Nacional Autonoma de Mexico

The *Drosophila* genus of the family Drosophilidae comprises around 1600 described species. These species differ in their geographic distribution and ecologies, and consequently, in ecological niches and lifestyles. As they evolved in different environments, species may differentially regulate their metabolism and behavior as they adapt to these local conditions. Here, we made a comparative study between *Drosophila lutzii,* a specialist, and sympatric *Drosophila simulans*, a generalist. *D. simulans* is a saprophytic generalist, with feeding based on rotting plants and fruits, while *D. lutzii* is a phytophagous specialist. We have found *D. lutzii* eggs, larvae, pupae and adults inside *Ipomoea sp.* flowers. This suggested a restricted diet, and thus, an interesting avenue for research in metabolism, in comparison to generalist species of flies. We found that freshly caught *D. lutzii* from the wild have higher carbohydrates levels, but similar lipid content, compared to sympatric freshly caught *D. simulans.* Consistent with a restricted diet and specialist lifestyle, *D. lutzii* flies survive less in culture in diets that differ in the amounts of carbohydrates. When fed diets with high sugar concentrations, contrary to *D. simulans*, they significantly accumulate them. Triglycerides levels also were differentially affected. in both species when fed with diets varying in sugar content. *D. lutzii* flies are significantly and dramatically less motile, but possess a circadian activity rhythm akin to *D. melanogaster or D. simulans*. These three species showed a differential feeding behavior when exposed to food with different amounts of sugar. Taken together, our results show that, in contrast to generalists, this specialist species, with more restricted habitat and feeding, is less capable of metabolic adjustments.

820V **A Novel Mistranslating tRNA Model in *Drosophila melanogaster* has Diverse, Sexually Dimorphic Effects** Joshua Isaacson[1], Matthew David Berg[2], Judit Villén[2], Christopher John Brandl[1], Amanda Jean Moehring[11]Biology, Western University, [2]Department of Genome Sciences, University of Washington

Transfer RNAs (tRNAs) are the adaptor molecules required for reading the genetic code and producing proteins. Transfer RNA variants can lead to genome-wide mistranslation, the misincorporation of amino acids not specified by the standard genetic code into nascent proteins. While genome sequencing has identified putative mistranslating transfer RNA variants in human populations, little is known regarding how mistranslation affects multicellular organisms. Here, we create a multicellular model of mistranslation by integrating a serine transfer RNA variant that mistranslates serine for proline (☐$tRNA^{Ser}_{UGG,G26A}$☐) into the *Drosophila melanogaster* genome. We confirm mistranslation via mass spectrometry and find that $tRNA^{Ser}_{UGG,G26A}$ misincorporates serine for proline at a frequency of ~0.6% per codon. $tRNA^{Ser}_{UGG,G26A}$ extends development time and decreases the number of flies that reach adulthood. While both sexes of adult flies containing $tRNA^{Ser}_{UGG,G26A}$ present with morphological deformities and poor climbing performance, these effects are more pronounced in female flies and the impact on climbing performance is exacerbated by age. RNA-seq performed on $tRNA^{Ser}_{UGG,G26A}$ flies indicate that mistranslation upregulates immune response genes. This work shows that mistranslating tRNA variants have diverse effects on *Drosophila melanogaster* and suggest that male and female flies differ in their ability to withstand the resulting proteotoxic stress. As humans have ~66 tRNA variants per person and several human diseases have been linked to mistranslation, this research advances our understanding of how tRNA variants affect human health.

821V **Adaptation to prolonged dietary iron depletion in *Drosophila melanogaster* (fruit fly): considerations for iron studies** Dawoud Usman[1,2], Farida Bashar[1,3], Saudatu Faruk[1,3], Kamaldeen Olalekan Sanusi[1,2], Muhammad Bashir Bello[1,4], Murtala Bello Abubakar[1,2], Kasimu Ghandi Ibrahim[1,2], Abdulllahi Yahya Abbas[5], Mustapha Umar Imam[1,61]Centre for Advanced Medical Research and Training, Usmanu Danfodiyo University, [2]Physiology, Usmanu Danfodiyo University Sokoto, [3]Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University Sokoto, [4]Department of Veterinary Microbiology, Usmanu Danfodiyo University Sokoto, [5]Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, [6]Department of Medical Biochemistry, Usmanu Danfodiyo University Sokoto

Iron deficiency is a common nutritional disorder that affects one third of the world. Iron depletion is an early indicator of the reduced iron stores that precedes iron deficiency. As such, chelating dietary iron is increasingly common for studying iron deficiency and evaluating therapies to iron excess. Due to the cost and time-consuming nature of mammalian studies and for sake of biological conservations, *Drosophila melanogaster* (fruit-fly) has become increasingly popular for investigating iron physiology. However, despite the extensive utility of iron depletion, its physiological responses at varied doses and durations remain poorly understood. Here, we depleted dietary iron across different durations of the fruit-fly life cycle using graded doses of Bathophenanthroline disulfonate (BPS). Most intriguingly, our dietary manipulation demonstrably elicited adaptations across distinct fly phenotypes. We report that iron content, fly mobility, survival, and pupal dimensions positively adapt to prolonged iron chelation while fly fertility adapts negatively. Interestingly, the threshold for adaptation is dependent on concentration of chelator, time and duration of exposure. Some form of compensation(s) or negative feedback relay(s) may be responsible. Taken together, our findings highlight considerations for dose and time in the study of iron deficiency, ferroptosis and the evaluation of therapies against several iron related conditions.

822V **Wingless signaling promotes lipid mobilization through signal-induced transcriptional repression** Rajitha Udakara Sampath Hemba-Waduge[1], Mengmeng Liu[1], Xiao Li[2], Xiahe Huang[3], Zhen Lin[4], Tzu-Hao Liu[1], Xianlin Han[5], Yingchun Wang[3], Jun-Yuan Ji[11]Biochemistry and Molecular Biology, Department of Biochemistry and Molecular Biology, Tulane University School of Medicine, Louisiana Cancer Research Center, [2]Lewis-Sigler Institute of Integrative Genomics, Princeton University, [3]Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, [4]Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, Louisiana Cancer Research Center, [5]Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio

Wnt/Wg signaling plays important roles in regulating normal development and energy metabolism in metazoans. Aberrant activation of Wnt signaling drives the tumorigenesis of various cancers such as colorectal cancer. However, the role of Wnt signaling in regulating intracellular lipid homeostasis remains poorly understood. We have recently reported that activation of Wg signaling in *Drosophila* larvae reduces the accumulation of triglycerides (TGs) in larval fat body. Reduced TG accumulation is accompanied with a significant increase of different types of free fatty acids (FFAs) in these larvae. To elucidate the mechanisms of how Wg signaling regulates lipid homeostasis, we carried out dual-isotope radio labelling experiments. We observed that rate of lipolysis in Wg activated cells is more prominent than lipogenesis, suggesting that enhanced lipolysis may contribute to reduced TG accumulation. Our transcriptomic and proteomic data revealed that many genes involved in lipogenesis, lipolysis, and fatty acid β-oxidation are downregulated by active Wg signaling in larval adipocytes. Furthermore, lipid accumulation defects in adipocytes caused by activated Wg signaling can be enhanced by depleting Lsd1, the key lipid droplet surface protein in *Drosophila,* but significantly rescued by ectopic expression of Lsd1 and Lsd2. Using the CUT&RUN assay, we identified a set of direct Wg target genes encoding key factors required for lipogenesis, lipolysis (such as lipid droplet-associated proteins), and fatty acid β-oxidation in mitochondria and peroxisome. These findings suggest that Wg signaling reduces intracellular TG accumulation by inhibiting lipogenesis and fatty acid β-oxidation but promoting lipolysis and lipid mobilization. Our results suggest that Wg signaling-induced transcriptional repression may play an important role in regulating intracellular lipid homeostasis.

823V **Females are more transgenerationally at risk of diseases related to parental zinc deficiency-induced glucose dysmetabolism in *Drosophila melanogaster* (fruit flies)** Kamaldeen Olalekan Sanusi[1,2], Dawoud Usman[1,2], Farida Bashar[2,3], Saudatu Faruk[2,3], Fati Ozioma Aliyu[1,2], Mustapha Umar Imam[2,41]Physiology, Usmanu Danfodiyo University, [2]Nutrigenomics, Centre for Advanced Medical Research and Training (CAMRET), UDUS, [3]Biochemistry and Molecular Biology, Usmanu Danfodiyo University, [4]Medical Biochemistry, Usmanu Danfodiyo University

Background: Parental environmental stress exposure such as malnutrition predispose offspring to later life diseases. Here, we modeled dietary zinc deficiency in fruit flies to probe its metabolic transgenerational effects. **Methods**: Male and female flies that developed on zinc-deficient diet (TPEN-supplemented at 50µM and 100µM) were crossed. The female flies were transferred to normal diet to lay eggs for 24 hours. F2 and F3 were generated by crossing F1 and F2 respectively with flies whose parents developed on normal diet. The F1-F3 offspring were maintained on normal diet for seven days after which they were anaesthetized and assessed for glucose, trehalose, glycogen, triglycerides, as well as fold changes in *DILP2* and *PEPCK* mRNA. **Results**: No significant difference was observed in the glucose and trehalose levels of F1 except in the female at 100µM which significantly increased. Moreover, a significant increase was observed in the levels of triglycerides and *DILP2* mRNA in the male and female F1. However, the levels of glycogen and *PEPCK* mRNA were significantly decreased in both male and female F1. In F2, no significant difference was observed in glucose levels, but a significant decrease was observed in the trehalose, glycogen and triglycerides levels. However, there was a

significant increase in the mRNA levels of *DILP2* and *PEPCK* in F2 male and female. Similarly, no significant difference in glucose levels of F3, except the females from grand maternal line which had significant increase. In addition, trehalose and glycogen levels decrease, but *PEPCK* mRNA increased in the F3 male and female. Interestingly, sex dependent effect was observed in the levels of triglycerides and *DILP2* mRNA, whereby there was an increase in the female but a decrease in the male F3. **Conclusions**: Combined maternal and paternal dietary zinc deficiency caused transgenerational glucose dysmetabolism through reduced trehalose and glycogen, and increased *PEPCK* mRNA in F3 offspring. This effect was prominent in female F3 as observed from increased levels of triglycerides and *DILP2* mRNA.

824V **Activation of Nrf2 in insulin-signaling impaired male *Drosophila melanogaster* improves resistance to paraquat and hydrogen peroxide** Jessica Alvarez[1], Juan Riesgo[21]UNAM, [2]UNAM INB

The Nrf2 signaling pathway functions to counteract oxidative stress, and we sought to understand whether its manipulation could enhance resistance to paraquat and hydrogen peroxide in adult insulin-compromised flies. One-week-old male and female flies with 1) heteroallelic mutations for InR (the fly homologue of the insulin receptor) or S6K (the fly homologue of ribosomal protein S6 kinase beta-1, under the control of TORC1) and 2) a heterozygous mutation for Keap1 (a negative regulator of CncC, Nrf2 in Drosophila), as well as wild-type controls with the same genetic background, were exposed to 3% hydrogen peroxide or 20 mM paraquat. We found that wild-type and insulin signaling-affected flies often showed similar resistance to both pro-oxidants, whereas males with the heterozygous Keap1 mutation, in addition to the insulin pathway mutations, had statistically significant enhanced survival to paraquat and hydrogen peroxide. Our results point to altered oxidative conditions in diabetic flies, where manipulation of the Nrf2 pathway may provide enhanced resistance to exogenous pro-oxidant agents, particularly in male flies.

825V **Fat body phospholipid state dictates hunger-driven feeding behavior** Kevin Kelly[1], Mroj Alassaf[1], Camille E Sullivan[1], Ava E Brent[1], Zachary H Goldberg[1], Michelle E Poling[1], Julien Dubrulle[2], Akhila Rajan[11]Basic Sciences, Fred Hutchinson Cancer Research Center, [2]Cellular Imaging Core, Fred Hutchinson Cancer Research Center

Diet-induced obesity leads to dysfunctional feeding behavior. However, the precise molecular nodes underlying diet-induced feeding motivation dysregulation are poorly understood. The fruit fly is a simple genetic model system yet displays significant evolutionary conservation to mammalian nutrient sensing and energy balance. Using a longitudinal high-sugar regime in *Drosophila*, we sought to address how diet-induced changes in adipocyte lipid composition regulate feeding behavior. We observed that subjecting adult *Drosophila* to a prolonged high-sugar diet degrades the hunger-driven feeding response. Lipidomics analysis reveals that longitudinal exposure to high-sugar diets significantly alters whole-body phospholipid profiles. By performing a systematic genetic screen for phospholipid enzymes in adult fly adipocytes, we identify Pect as a critical regulator of hunger-driven feeding. Pect is a rate-limiting enzyme in the phosphatidylethanolamine (PE) biosynthesis pathway and the fly ortholog of human PCYT2. We show that disrupting Pect activity only in the *Drosophila* fat cells causes insulin resistance, dysregulated lipoprotein delivery to the brain, and a loss of hunger-driven feeding. Previously human studies have noted a correlation between PCYT2/Pect levels and clinical obesity. Now, our unbiased studies in *Drosophila* provide causative evidence for adipocyte Pect function in metabolic homeostasis. Altogether, we have uncovered that PE phospholipid homeostasis regulates hunger response.

826V **Biotransformed citrus extract improves intestinal barrier integrity and reduces oxidative damage in female *Drosophila melanogaster*** Nadiia Sadova[1], Alice König[1,2], Julian Weghuber[1,21]University of Applied Sciences Upper Austria, [2]FFoQSI – Austrian Competence Centre for Feed and Food Quality, Safety & Innovation

Citrus bioflavonoids are widely studied for their potential to strengthen the intestinal barrier and to reduce oxidative damage. However, there is limited knowledge on the bioavailability of flavonoids and its impact on the antioxidant capacity of these nutrients in living organisms. For this study, we tested potential health-promoting effects of aqueous extracts of *Citrus aurantium* var. *amara L., Citrus sinensis, Citrus paradisi* powder (AQE) rich in naringin and hesperidin and its bio-transformed version submitted to citric acid hydrolysis and fermentation (FermCAE) rich in naringenin and hersperitin in the in vivo model *Drosophila melanogaster*. To test the influence of AQE and FermCAE on intestinal barrier integrity, 5 d. o. female w^{1118} fruit flies were challenged with dyed 5% dextran sodium sulphate solution for 7 days. Dead flies, as well as those that expressed Smurf phenotype were scored throughout 3 independent experiments. Furthermore, oxidative stress challenge was performed. Therefore, 30 mM iron (II) sulphate was added to the solid food and introduced to the test subjects for 3 biochemical assays (ROS level, MDA content and metabolic activity), mortality and climbing activity tests, respectively. In both sets of experiments *D. melanogaster* were divided into control groups (no stressor or flavonoids), stressor only group and treatment groups with stressor and one of the tested extracts (AQE,

FermCAE) in 2 different concentrations (2.5%, 5% (v/w)). Our results suggest that the biotransformation of the glycosides naringin and hesperidin into the aglycons naringenin and hesperitin has significantly improved their protective effect against intestinal barrier damages in a dose-dependent manner. Furthermore, we observed significant reduction of ROS level in flies stressed with iron sulphate and treated with citrus flavonoid extracts, with most antioxidant effect observed in the group treated with 5% FermCAE. Significant positive impact of AQE and FermCAE was also observed in terms of survivorship and climbing activity of fruit flies exposed to oxidation-inducing stressor. We conclude that supplementation with fermented citrus flavonoids extract improves survivorship, intestinal integrity, and climbing activity in *D. melanogaster* upon various stressors.

827V **Tissue-specific requirements of key autophagy genes in adult fruit fly lifespan regulation** Mariah Bierlein[1], Joseph Charles[1], Polisuk-Balfour Trevor[1], Micaela Rice[1], Jacklyn Zvonar[1], Drake Pohl[1], Lindsey Winslow[1], Brennah Wasie[1], Sara Deurloo[1], Jordan Van Wert[1], Britney Williams[1], Gabrielle Ankney[1], Zachary Harmon[1], Erica Dann[1], Anna Azuz[1], Alex Guzman-Vargas[1], Elizabeth Kuhns[1], Felix Amissah[1], Thomas P. Neufeld[2], Michael B. O'Connor[2], Changqi C. Zhu[1]1Ferris State University, [2]University of Minnesota

Aautophagy, a lysosome-based eukaryotic cellular degradation system, has previously been implicated in lifespan regulation in different animal models through different tissue types. To better understand the functions of the key autophagy genes in adult fly lifespan regulation, we tested if the key autophagy genes such as Atg1, Atg5, Atg9, or Atg18 play important roles in adult fruit fly (*Drosophila melanogaster*) lifespan regulation through adult fly muscle, fat body, and gut stem cells. In this report, we show that knockdown of the transcripts of Atg1, Atg5, Atg9, or Atg18 in adult fly muscle does not alter the overall lifespan, but lifespan reduction phenotype becomes apparent through the knockdown of the transcripts of Atg1 or Atg18 in adult fly adipocytes or gut stem cells. Over-expression of wild type Atg1 in adult fly muscle or adipose tissue reduces lifespan. This lifespan reduction phenotype is independent of the function of Atg9 or Atg18, but it correlates with the high levels of ubiquitinated protein aggregates from the Atg1 over-expressing tissues. Our research data presented here have highlighted the important functions of the key autophagy genes in adult fly adipose tissue and midgut stem cells for lifespan regulation and their undiscernible roles in adult fly muscle.

828V **Peppers in Diet: Genome-Wide Transcriptome and Metabolome Changes in *Drosophila melanogaster*** Carlos Lopez-Ortiz, Mary Edwards, Purushothaman Natarajan, Padma Nimmakayala, Umesh K ReddyWest Virginia State University

The habanero pepper (*Capsicum chinense*) is an increasingly important spice and vegetable crop worldwide because of its high capsaicin content and pungent flavor. Diets supplemented with the phytochemicals found in habanero peppers might cause shifts in an organism's metabolism and gene expression. Thus, understanding how these interactions occur can reveal the potential health effects associated with such changes. We performed transcriptomic and metabolomic analyses of *Drosophila melanogaster* adult flies reared on a habanero pepper diet. We found 539 genes/59 metabolites that were differentially expressed/accumulated in flies fed a pepper versus control diet. Transcriptome results indicated that olfactory sensitivity and behavioral responses to the pepper diet were mediated by olfactory and nutrient-related genes including gustatory receptors (*Gr63a*, *Gr66a*, and *Gr89a*), odorant receptors (*Or23a*, *Or59a*, *Or82a*, and *Orco*), and odorant-binding proteins (*Obp28a*, *Obp83a*, *Obp83b*, *Obp93a*, and *Obp99a*). Metabolome analysis revealed that campesterol, sitosterol, and sucrose were highly upregulated and azelaic acid, ethyl phosphoric acid, and citric acid were the major metabolites downregulated in response to the habanero pepper diet. Further investigation by integration analysis between transcriptome and metabolome data at gene pathway levels revealed six unique enriched pathways, including phenylalanine metabolism; insect hormone biosynthesis; pyrimidine metabolism; glyoxylate, and dicarboxylate metabolism; glycine, serine, threonine metabolism; and glycerolipid metabolism. In view of the transcriptome and metabolome findings, our comprehensive analysis of the response to a pepper diet in Drosophila have implications for exploring the molecular mechanism of pepper consumption.

829V **Characterizing Muscle Disuse in *Drosophila*** Carthic Rajagopalan, Jodi Protasiewicz, Robert J. WessellsPhysiology, Wayne State University

Sedentary behavior is a leading factor in the etiology of many age-related diseases, as well as a key contributing factor to loss of mobility and independence in the aging population. While these consequences can be avoided in many patients by increasing activity levels, a substantial subset of the population is unable to perform chronic exercise due to illness, injury or living situations that enforce long sedentary periods. Thus, identification of single-molecule mimetics that can counteract the deleterious effects of prolonged disuse on muscle structure and function could have a transformative

impact on healthcare for elderly and/or disabled patients. While much is known about the molecular pathways induced by chronic exercise, there remains a knowledge gap about the long-term effects of upregulation of exercise-mediating pathways on healthspan in chronically sedentary individuals. Here, we characterize a novel *Drosophila* model for long-term sedentary behavior and provide definitive evidence of the efficacy of multiple putative exercise mimetics in combatting the effects of enforced sedentary behavior on skeletal muscle. We generated a model of muscle disuse via enforced restraint in order to characterize these effects. Fluorescent imaging of muscle structure and physical performance assays revealed a stark contrast in the overall muscle health of unrestrained versus restrained flies. Flies under restraint possess visibly damaged muscle fibers and perform significantly worse in terms of endurance, climbing speed, and lifespan as compared to unrestrained flies. These findings establish a novel restraint model in Drosophila with which to base future work on. Having characterized this model, we are currently testing the potential of rescuing muscle health of restrained flies through the use of exercise mimetics.

830T **Does developmental ethanol exposure trigger neurodegeneration? The interaction between ethanol and mutations causing neurodegneration in Drosophila.** Navneet SangheraBiological 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 also 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, and that DAE results in changes in *Dmtn* expression. In addition, we find 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 cause issues with movement, power, senses, and cognition. Our data suggest that DAE may cause neurodegeneration (and may target known pathways involved in neurodegeneration). We are therefore performing a survey of the interaction between DAE and Drosophila models of neurodegeneration. We will present the results of testing the ethanol sensitivity of a variety of different neurodegenerative mutants associated with Amyotrophic Lateral Sclerosis, Alzheimer's Diseases, and Parkinson Disease. This research was supported by grants from the National Institutes of Health National Institute of General Medical Sciences (5SC3GM103739) and National Institute on Alcohol Abuse and Alcoholism (1R15AA027678), and a Project Development Grant from the California State University Program for Education and Research in Biotechnology.

831T **Synaptic heterogeneity among the compartments ofaxon terminals** Hongyang Wu[1], Sayaka Eno[1], Kokoro Saito[1], Shu Kondo[2], Nobuhiro Yamagata[3], Hiromu Tanimoto[31]Graduate School of Life Sciences, Tohoku University, [2]Department of Biological Science and Technology, Tokyo University of Science, [3]Tohoku University

Structures of individual synapses should ideally be tuned to precise synaptic transmission and plasticity. Regulation of active zone heterogeneity and underlying molecular mechanisms in the central nervous systemare largely unknown. We here focus on intracellular assemblies of active zones, presynaptic vesicle release sites which facilitate the release of synaptic vesicles. By applying the split-GFP based approach for a cell-type specific visualization of the active zone main component, Bruchpilot (Brp), we demonstrate the heterogeneity of active zones along the different compartments of Kenyon cell (KC). Visualization of endogenous Brp in the APL and DPM neurons, identified single pairs of neurons synapsing on the entire mushroom body, revealed compartmental heterogeneity strikingly similar to the pattern of KCs. We identifieddifferent signals that regulate this heterogeneity. Our results suggest that multiple layers of modulatory signals spatially regulate the size of active zones.

832T ***Investigating the role of ATM kinase in synapse development*** Matthew J Taylor, Richard TuxworthCancer and Genomic Sciences, University of Birmingham

Ataxia-telangiectasia (A-T) is an early-onset neurodegenerative disease caused by mutations in a key player of the DNA damage response (DDR): ATM kinase. A-T is characterised by radiosensitivity, immunodeficiency, and cerebellar degeneration, although it is unclear why cerebellar neurons are particularly vulnerable to ATM deficiency. ATM is also activated by reactive oxygen species (ROS) independently of the DDR, where it has distinct targets, such as the autophagic machinery. Further evidence suggests ATM has unique roles in neurons; it colocalises with synaptic vesicles and is required for long-term potentiation.

Like mammalian ATM, *Drosophila* ATM (dATM) is important for the response to double-strand breaks, and others have demonstrated the importance of dATM signalling in adult glial cells. However, little is known about the neuronal role of dATM, particularly during development. ROS signalling positively regulates synapse growth in *Drosophila*, although excessive ROS promotes neurodegeneration through excitotoxicity; it is unclear how this balance is mediated.

We are investigating the role of dATM during neurodevelopment using the larval neuromuscular junction (NMJ). *dATM* mutants show a marked decrease in NMJ size, number of active zones and bouton count. This deficit is recapitulated by presynaptic knockdown using neuronal RNAi, but dATM is not required postsynaptically nor in the peri-synaptic glia. This appears to be independent of DDR signalling, since neuronal knockdown of upstream or downstream DDR components do not affect NMJ development. The phenotype mimics that seen with knockdown of autophagy genes, and we found that upregulation of autophagy rescued the growth of the NMJ. Neuronal knockdown of dATM also sensitises larvae to excitotoxicity, without detectable changes in DNA damage levels.

We generated a functional *superfolder*GFP-dATM fusion by CRISPR/Cas9, which complements *dATM* mutants, rescues the NMJ phenotype, and restores phosphorylation of the ATM-target, ɣH2Av, after irradiation. *SuperfolderGFP-d*ATM localises to foci in presynaptic boutons, which intriguingly may be synaptic vesicles. Our data suggest that dATM may transduce presynaptic ROS levels, responding to increased ROS by activating the autophagic machinery to induce synaptic growth, while protecting the cell from activity-induced excitotoxicity.

833T **Notch signaling positively regulates early temporal factor expression and timing of MB neurogenesis termination** Kendall Branham, Chhavi Sood, Sarah SiegristUniversity 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 a set developmental timeline. In Drosophila NSCs known as neuroblasts (NBs), 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 and autism spectrum disorders, or overproliferate leading to macrocephaly and tumors. We know programs to control timing of proliferation and elimination of NSCs exist, but many elements of temporal cassettes are still unclear. What genes may be upstream to regulate known temporal cassettes to control when certain progeny are produced have not been fully identified leaving a gap in our understanding. To address these questions, we carried out a large scale RNAi screen aimed at identifying genes required for NSC elimination. We identified Notch and its ligand, Delta. When Notch pathway activity is reduced in NSCs, we found premature elimination of an important subset of neuroblasts called the mushroom body neuroblasts (MBNBs). These MBNBs produce the neurons responsible for formation of the evolutionarily conserved structure called the mushroom body (MB), which is involved in olfaction based learning and memory. MBNBs with reduced Notch pathway activity also lead to defects in MB structure. Furthermore, we determined that temporal patterning is disrupted primarily through loss of early temporal factor expression. In this work, we find that cell signaling pathways that involve the receptor Notch and its ligand Delta function to regulate NB proliferation in Drosophila melanogaster by regulating early temporal factor expression.

834T **Clock mRNAs and proteins are organized into cytoplasmic membraneless ribonucleoprotein condensates** Ye Yuan[1], Dunham Clark[2], Rebecca Tran[2], Swathi Yadlapalli[11]Program in Cellular and Molecular Biology, University of Michigan, [2]College of LSA, University of Michigan

Circadian clocks are cell autonomous timekeepers that regulate ~24-hour oscillations in the expression of many genes leading to rhythms in physiology and behavior. All 24-hour circadian clocks are controlled by negative feedback loops that are enabled by biochemically controlled time delays, e.g., long delays (~8-h) between core clock mRNA and protein accumulation. However, where and how clock mRNAs are organized in clock neurons and whether subcellular organization of mRNAs is critical for ~24-h circadian rhythms is unknown. Using *Drosophila* clock neurons as a model system, my recent work has revealed for the first time that clock mRNAs and proteins assemble into ribonucleoprotein (RNP) condensates in the perinuclear cytoplasmic region to control critical time delays between clock mRNA and protein accumulation and enable circadian rhythms with ~24-h periodicity.

Specifically, I discovered that mRNAs of *period* (*per*), a core clock gene, and its kinase *doubletime* (*dbt*, a homolog of mammalian CK1) are organized into membraneless RNA condensates (~700 nm) in the perinuclear cytoplasm of *Drosophila* clock neurons. *Dbt* and *per* mRNAs localized to the condensates were translationally active; DBT kinase accumulated at the surface of *per*/*dbt* ribonucleoprotein (RNP) condensates and PER protein was phosphorylated by DBT

within these condensates. The role of *per/dco* RNP condensates in delayed accumulation of PER protein is highlighted by the observation that DCO knockdown led to immediate accumulation of unphosphorylated PER protein in the nucleus resulting in a complete loss of circadian rhythms. Further, SLMB, an E3-ubiquitin ligase required for PER degradation and a homolog of mammalian b-TrCP, was localized to a single focus at the centrosome, indicating that phosphorylated PER protein was transported from the RNP condensates to the centrosome for ubiquitination and rapid degradation. *Per/dbt* RNP condensate assembly was promoted by the RNA-binding protein ENCORE. Loss of ENCORE disrupted *per*/*dbt* RNP condensates, caused prolonged PER protein degradation, and led to longer (~26-h) circadian rhythms. Thus, our studies reveal how clock ribonucleoprotein condensates enable precise spatiotemporal control of phosphorylation and degradation of a core clock protein to ensure ~24-h circadian rhythms. Insights from our studies will be broadly relevant as DBT/CK1 and SLMB/bTrCP are common to many developmental pathways such as Wnt and Hedgehog.

835T **Investigating interactions between Tsp42Eg and PI(4,5)P_2 at the synapse in *Drosophila tsp42Eg* mutants** Stephanie M Mullen, Emily L Hendricks, Faith LW LieblBiological Sciences, Southern Illinois University Edwardsville

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) is a plasma membrane lipid known to regulate vesicle trafficking, actin dynamics, and ion-channel currents at synapses. PI(4,5)P_2 is decreased in post-mortem examinations of Alzheimer's disease brain tissue and there is a negative correlation between Aβ oligomers at the synapse and PI(4,5)P_2. Aβ oligomers form from the amyloidogenic processing of amyloid precursor protein (APP) by β- and γ- secretases, generating Aβ monomers that are exocytosed before forming the extracellular oligomers observed in the pathogenesis of Alzheimer's disease. Tetraspanins (Tsps), transmembrane proteins that regulate the spatiotemporal distribution and interactions of proteins at the plasma membrane, associate with ADAM10 and γ-secretase, both of which are involved in APP processing. The specific role of Tsps in regulating protein interactions at the synapse, however, has not been well-characterized. *tsp42Eg* mutants exhibit decreased levels of PI(4,5)P_2 at the synapse, increased Aβ levels, and decreased amyloid precursor protein like (APPL). To investigate the relationship between Tsp42Eg and PI(4,5)P_2, we used two compounds, Copanlisib and m-3M3FBS, which increased PI(4,5)P_2 levels in controls. Copanlisib inhibits PI3K, an enzyme that converts PI(4,5)P_2 into PIP_3, while m-3M3FBS activates PLC, an enzyme that cleaves PI(4,5)P_2 into DAG and IP3. Lifetime treatment with these compounds in *tsp42Eg* mutants produces a stepwise decrease in PI(4,5)P_2 levels with m-3M3FBS producing a minimal decrease in PI(4,5)P_2 and Copanlisib a more dramatic decrease. We are currently examining the possibility that modulating PI(4,5)P_2 levels in *tsp42Eg* mutants will impact synaptic levels of APPL and Aβ.

836T **Regulation of cell number in the *Drosophila* visual system** Jennifer Malin, Yen-chung Chen, Felix Simon, Evelyn Keefer, Claude DesplanDepartment of Biology, New York University

Neurons must be generated in the correct proportions so that they can scale their arbors and sample the appropriate number of inputs. Distal medulla (Dm) neurons are a class of 20 visual system interneurons that are present in highly regular numbers within the brain. These neurons are born from a crescent-shaped neuroepithelium called the Outer Proliferation Center, or OPC. The OPC is spatially subdivided into discrete compartments based on the non-overlapping expression of various transcription factors and growth factors. During development, the neuroepithelium is converted into stem cells that sequentially express a series of transcription factors. Previous work from the lab showed that the integration of spatially and temporally controlled transcription factors promotes Dm neuron fate, but it was unknown how this interplay led to the generation of each cell type in the correct proportions.

Through lineage tracing and genetics experiments, we determined the relationship between neuroepithelial compartment size and cell number. We found that abundant neural types (such as Dm3- 750 cells per hemibrain) are born from the entire OPC, while less numerous types (i.e. Dm4- 40 cells per hemibrain) are born from discrete domains within the OPC. However, this finding failed to explain more granular regulation of cell number (e.g. two subtypes containing 40 and 120 cell types, respectively, appeared to derive from the same compartment). We therefore, inhibited apoptosis or increased stem cell proliferation (by expanding the size of the temporal transcription factor window) and found that these manipulations only caused minor increases in cell number across cell types, indicating that the canonical mechanisms of death and proliferation were not major players in this process. However, our lab identified additional spatial factors that appear to subdivide the OPC into smaller compartments, which appears to account for most of the differences in cell number. Our work suggests that spatial patterning allocates differently sized pools of stem cells for the generation of different proportions of neurons, providing a novel mechanism for regulating cell number.

837T **A hierarchical, combinatorial transcription factor code for leg sensory neurons revealed by single-cell RNA-sequencing** Ben Hopkins, Olga Barmina, Artyom KoppEvolution & Ecology, UC Davis

To respond to the world around them, animals rely on input from a network of sensory organs. Distinct classes of sensory organ, each composed of multiple cell types, appear specialised to detect specific stimuli. This diversity of cell types, both within and between organs, raises the dual questions of how this diversity is generated during development and what makes these cell types distinct from one another? To address these questions, we performed single-cell RNA-seq on a region of the pupal male *Drosophila* leg that displays a variety of functionally and structurally distinct sensory organs. Using this data, we identify and validate a transcription factor (TF) code that distinguishes between sensory neuron populations. Hierarchical, first-order differences distinguish between neurons involved in mechano- (*Ets65A*) and chemo- (*pros*) sensation. A layer below, second-order differences describe subtypes within these classes: combinatorial expression of *vvl* and *eyg* distinguishes between mechanosensory neurons innervating mechanosensory bristles, campaniform sensilla, and chemosensory bristles; while *acj6, fkh,* and *nvy* distinguish between four transcriptomically distinct gustatory receptor neuron (GRN) populations. We identify the same four GRN populations in the Fly Cell Atlas adult data. Therefore, despite well documented variation in sensitivity and responsiveness between chemosensory taste bristles, our work suggests that these organs are largely innervated by a minimal number of four distinct GRN classes, each defined by a unique combination of TFs. An additional level of regulation is likely layered on top of this core regulatory program to allow the same GRN class in different bristles to express different gene repertoires and, by extension, to exhibit divergent sensitivities to tastants. This regulatory layer likely takes in positional and sex differentiation signals. Indeed, our data suggest that *fru* and *dsx* are restricted to subsets of neurons within each GRN class. We link each GRN class defined by the TF code to distinct repertoires of receptors and membrane channels and, in three cases, to known GRN subtypes: the *ppk23*$^{+}$ male- and female-pheromone-sensing GRNs and *Ir52a-d*$^{+}$ courtship neurons. This leaves the identity of the *fkh*$^{+}$ GRN as a mystery. Finally, I show that homologous accessory cell types between different organ classes express distinct gene repertoires, providing a non-neuronal route to between-organ variation in sensory capabilities.

838T **Conserved transcription factors Eyeless and Scarecrow regulate the specification of olfactory navigation input neurons** Alexa Gonzalez, Aisha Hamid, Mubarak H. SyedBiology, The University of New Mexico

The molecular mechanisms responsible for the generation of diverse neural types populating the brain are not completely understood. The proper production of diverse neural types in *Drosophila* is essential for the formation of unique circuits that mediate various essential behaviors, including olfactory navigation. One neural stem cell type in *Drosophila*'s central brain, denoted as type II neural stem cells (type II NSCs), divide asymmetrically to generate intermediate neural progenitors (INPs) that amplify and diversify most of the neural types populating the adult *Drosophila* brain. The temporally expressed genes in type II NSCs and INPs are thought to regulate the production of diverse neural types. However, how type II NSCs and INPs together generate distinct neural types in the central complex is currently unknown. This study focuses on the INP specific transcription factors and their role in the specification of a unique neural type called ventral fan-shaped body (vFB) neurons, which are the tangential input neurons of the olfactory navigation circuit. Our preliminary data shows that INP specific knockdown of Eyeless results in a complete loss of vFB neurons. We also found that late factor Scarecrow is essential for the proper specification of the vFBs, thus suggesting a developmental role of late INP factors to produce vFB neurons properly. Altogether, our results suggest that temporally expressed transcription factors in INPs, Eyeless and Scarecrow regulate the specification of olfactory navigation input neurons. In the future, we will test if Eyeless and Scarecrow regulate the olfactory navigation behavior.

839T **Targeted DamID identifies novel transcriptional targets of Alk signalling in *Drosophila* neuroendocrine cells.** Sanjay Kumar Sukumar[1], Vimala Antonydhason[1], Linnea Molander[1], Jawdat Sandakly[2], Ganesh Umapathy[1], Tafheem Masudi[1], Patricia Mendoza-Garcia[1], Margret Shirinian[2], Ruth Palmer[11]Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine, [2]Department of Experimental Pathology, American University of Beirut

Anaplastic lymphoma Kinase (ALK) is a receptor tyrosine kinase that is implicated in various human cancers including neuroblastoma and lung cancer. In *Drosophila*, Alk is expressed in the developing embryonic visceral mesoderm, CNS and at neuromuscular junctions. Numerous roles for Alk have been described in the CNS, but the molecular details are poorly understood. To gain mechanistic insight, we set out to identify transcriptional targets of Alk signalling in the larval CNS using the transcriptional profiling technique known as Targeted DamID (TaDa). TaDa was employed in larval CNS tissues, while simultaneously genetically manipulating Alk signalling output. Analysis of the resulting TaDa datasets identified transcriptional regulation of genes expressed in neuroendocrine cell clusters by Alk. Further integration with bulk/scRNA-seq and proteomics datasets from larval brains in which Alk signalling was manipulated, identified a previously uncharacterized *Drosophila* neuropeptide encoded by *CG4577*, which we named Sparkly (Spar). To further characterize Spar, we generated anti-Spar antibodies showing that Spar is expressed in a subset of Alk-positive neuroendocrine

cells in the developing larval CNS. In agreement with our TaDa analysis, inhibition of Alk signalling with a dominant-negative Alk transgene led to decreased Spar protein levels, while overexpression of the *Drosophila* Alk ligand Jeb resulted in increased levels of Spar protein in the larval CNS. Further in-depth analysis of *spar* mutant flies showed a significant reduction in life-span, and behavioral phenotypes including defects in activity, sleep and circadian rhythm. The molecular function of Spar in the context of Alk signalling in *Drosophila* larval CNS is being studied in detail and our recent findings will be presented. These findings of a novel role for Alk in regulation of endocrine function in *Drosophila* are in agreement with the previously reported role of Alk in the hypothalamic-pituitary-gonadal axis in mice (Witek et al., 2015). Taken together, our findings suggest an evolutionarily conserved role of Alk signalling in the regulation of neuroendocrine function.

840T **Anteroposterior segmental specialization of the nervous system: The homeotic modulation of a sexually dimorphic circuit** Nicole Leitner, Yehuda Ben-ShaharBiology, Washington university in St. Louis

Segmental specializations of neuronal morphology and physiology along the anteroposterior adult brain axis play a fundamental role in locomotion, cognition, and behavior. However, the genetic and molecular mechanisms that drive segment-specific differentiation of homologous neurons remain poorly understood. Therefore, we propose the hypothesis that segmental neuronal specializations are driven via segment specific interactions between canonical neuronal differentiation pathways and homeotic segment identity factors. To test this hypothesis, we investigate how genetic interactions between the homeotic factors that determine thoracic segment identity and the canonical neuronal sex determination pathway drive the segmental specializations of pheromone-sensing neurons in adult legs that express the ion channel *pickpocket23* (*ppk23*). Specifically, we focus on one important morphological feature, the axonal midline crossing decision. We show that the axons of *ppk23*-expressing neurons cross the midline of the of the ventral nerve cord (VNC) in males but not in females, while those in the meso- (T2) and metathoracic (T3) legs do not cross the midline in both sexes. Furthermore, we present evidence that this T1 segment specific polymorphism is regulated via an interaction between the non-cell-autonomous action of the homeotic gene *Sex combs reduced* (*Scr*) and the cell-autonomous action of the male specific neuronal differentiation factor *fruitless* (*fru*). Based on these data, we propose a model which stipulates that *Scr* promotes axonal midline crossing in the prothoracic VNC by unmasking fru^{M}-dependent regulation of cell-autonomous neuronal signaling pathways that regulate the axonal midline crossing decision.

841T **Ssdp Influences Neurodevelopment and Autism-like Behaviors in *Drosophila melanogaster*** Safa Salim, Amira Alwa, Sadam Hussain, Foysal Ahammad, Swetha Gowda, Mohammad FarhanHamad Bin Khalifa University

1p32.3 microdeletion/duplication is associated with many neurodevelopmental disorders-like phenotypes, including developmental delay, intellectual disability, autism, macro/microcephaly, and dysmorphic features. The 1p32.3 chromosomal region harbors several genes critical for development, however, they have not been functionally validated or characterized. One of the genes in the 1p32.3 microdeletion region is the single-stranded DNA binding protein 3 (*SSBP3*), and its *Drosophila melanogaster* ortholog is called sequence-specific single-stranded DNA-binding protein (Ssdp). We used Ssdp knockdown and mutant male *D.melanogaster* models and assessed their neurodevelopment, physiological function, and autism-associated behaviors. We show that SSBP3 and Ssdp are expressed in excitatory neurons in the brain. Ssdp deficiency in brain causes a selective decrease in glial cells, with no effect on neuronal numbers but a significant increase in axon and dendrites proportion. Ssdp functions via the canonical Wnt signaling pathway in brain development in *D. melanogaster.* We show that Ssdp mutants have increased brain oxidative stress and defects in neuronal mitochondrial morphology. Genes involved in mitochondrial fission and fusion are upregulated in Ssdp mutant brains. Flies with reduced levels of Ssdp show heightened anxiety-like behavior, altered decisiveness, defective sensory perception and habituation, abnormal social interaction, and feeding defects. We further show that pan-neuronal overexpression of human SSBP3 partially shows gain-of-function behavioral phenotypes. Our findings support that the SSBP3 is a critical gene in the 1p32.3 microdeletion/duplication region and is associated with autism-like behavioral abnormalities.

842T **Differentiation signals from glia are fine-tuned to set neuronal numbers during development** Anadika R Prasad, Inês Lago Baldaia, Matthew P Bostock, Zaynab Housseini, Vilaiwan M FernandesCell and Developmental Biology, University College London

Neural circuit formation and function require that diverse neurons are specified in appropriate numbers. Known strategies for controlling neuronal numbers involve regulating either cell proliferation or survival. We used the *Drosophila* visual system to probe how neuronal numbers are set. Photoreceptors from the eye-disc induce their target field,

the lamina, such that for every unit eye there is a corresponding lamina unit (column). Although each column initially contains ~6 post-mitotic lamina precursors, only 5 differentiate into neurons, called L1-L5; the 'extra' precursor, which is invariantly positioned above the L5 neuron in each column, undergoes apoptosis. Here, we showed that a glial population called the outer chiasm giant glia (xg^O), which resides below the lamina, secretes multiple ligands to induce L5 differentiation in response to epidermal growth factor (EGF) from photoreceptors. By forcing neuronal differentiation in the lamina, we uncovered that though fated to die, the 'extra' precursor is specified as an L5. Therefore, two precursors are specified as L5s but only one differentiates during normal development. We found that the row of precursors nearest to xg^O differentiate into L5s and, in turn, antagonise differentiation signalling to prevent the 'extra' precursors from differentiating, resulting in their death. Thus, an intricate interplay of glial signals and feedback from differentiating neurons defines an invariant and stereotyped pattern of neuronal differentiation and programmed cell death to ensure that lamina columns each contain exactly one L5 neuron.

843T **Evaluating the role of serotonin receptors in experience dependent critical period plasticity** Ahana Mallick[1], Jacob M Epstein[1], Hua Leonard Tan[1], Andrew Dacks[2], Quentin Gaudry[11]Biology, University of Maryland, College Park, [2]Biology, West Virginia University

Critical periods (CPs) are brief time windows in the early life of an organism when their neuronal circuits exhibit astounding levels of circuit reprogramming and refinement in response to various sensory experiences. This form of neuroplasticity is regulated by the onset of sensory experiences. Neuromodulators like serotonin(5-HT) play a central role in inducing plasticity during critical periods. However, the exact cellular and molecular mechanism of serotonin signaling in this form of plasticity remains unclear. Chronic exposure to high odor concentrations during the CP induces odor-specific structural plasticity in the *Drosophila* antennal lobe (AL) olfactory circuit. Here we investigate the neuromodulatory mechanisms by which 5-HT regulates such plasticity. Previous studies have shown that chronic exposure to 5% CO_2 during the CP leads to activity dependent increase in the volume of the CO_2 specific V-glomerulus of the antennal lobe. We find that blocking the release of synaptic 5-HT in the antennal lobe prevents the onset of structural plasticity in the V-glomerulus following chronic exposure to CO_2 during the CP. We also identify that serotonin receptors (5-HTR) 5-HT1B, 5-HT2B and 5-HT7 are required to induce critical period plasticity in the antennal lobe. Knocking down 5-HT7 receptors in a specific subset of pan-glomerular inhibitory local interneurons (LNs) is sufficient to block critical period plasticity in the antennal lobe. This indicates that 5-HT signaling plays a crucial role in modulating global inhibitory circuits in the antennal lobe during the CP. Further, knocking down 5-HT2B in olfactory sensory neurons (OSNs) is also sufficient to block critical period plasticity in the V-glomerulus. Taken together, these results indicate that 5-HT signaling might play a role both locally as well as in the surrounding glomeruli via interneurons to facilitate critical period structural plasticity in the V glomerulus.

844T **Investigating the localization and function of laminin and dystroglycan in *Drosophila* wrapping glia development** Katherine V Clayworth, Vanessa J AuldZoology, University of British Columbia

Peripheral nervous system (PNS) health is largely dependent on proper glial cell functioning during development. Myelinating and non-myelinating Schwann cells (MSCs and NMSCs, respectively) are glial cells in the PNS that ensheathe and protect axons. Communication between Schwann cells and the extracellular matrix (ECM) is essential for PNS development. The ECM protein laminin, and its receptor dystroglycan [Dg; part of the dystrophin-glycoprotein complex (DGC)], are important for MSC development, however very little is known about the mechanisms underlying the role of laminins, Dg, and the DGC in NMSC development. We use developing *Drosophila* wrapping glia (WG), which ensheathe axons similarly to NMSCs, as a model to study the role of laminin/Dg in NMSC development. We found strong expression of LanA (one of two laminin alpha subunits in *Drosophila*), around WG. Wing blister, the other laminin alpha subunit, is not strongly expressed the peripheral nerve, indicating that the LanA-containing isoform is the primary laminin isoform expressed. Knockdown of LanA in WG eliminated LanA expression around WG and caused WG swellings, confirming that LanA is expressed by WG. Preliminary data suggests that LanA is most often found at the adaxonal WG membrane (between WG and axons), rather than the abaxonal WG membrane (between WG and its adjacent glial layer, subperineurial glia). Furthermore, LanA appears preferentially associated with motor axons versus sensory axons. These results potentially indicate a form of WG polarization and differential axonal wrapping, features that have not been well understood in WG thus far. We found the laminin receptor Dg is also expressed on WG membranes, however there are three isoforms of Dg and their individual expression patterns in the PNS are unknown. Using CRISPR and phiC31-mediated recombination, we tagged alternatively spliced exons of Dg and identified differential expression of Dg isoforms in the layers of the peripheral nerve. Knockdown of Dg and dystrophin (a component of the DGC), leads to WG ensheathment failure, indicating that Dg and dystrophin are important for WG development. We are investigating other

DGC components (e.g., dystrobrevin, syntrophins) to determine the composition of the DGC in WG. Due to the highly conserved nature of laminins and DGC proteins, our results have implications for NMSC development—thus improving our understanding of the factors underlying PNS development in all animals.

845T **Regulation of glial septate junction proteins by microRNA-184** Sravya Paluri, Vanessa AuldLife Sciences Institute, University of British Columbia

Glial cells are crucial for many processes including providing structural support to neurons and for maintenance of the blood-brain/nerve-barrier (BBB). Permeability barriers are formed by septate junctions (SJ) in *Drosophila* to restrict diffusion of molecules across tissues. SJs comprise of many proteins including: NeurexinIV (NrxIV), Macroglobulin-complement-related (Mcr), kune-kune (kune) and sinuous (sin). Barriers formed at the convergence of three SJ form the tricellular junction (TCJ), which includes proteins like Anakonda, M6 and Gliotactin (Gli). A specific class of glia, the subperineurial glia (SPG), form auto-SJs with themselves and each other to create the BBB around the nervous system. Loss of a single core SJ or TCJ protein compromises the BBB leading to paralysis and lethality. While these junctions have been extensively studied in other tissues, their regulation in the nervous system is less studied. microRNA-184 (miR-184) is predicted to target a wide range of *Drosophila* SJ and TCJ mRNAs. miR-184 targets SJ proteins in epithelia, but its role in the nervous system is not known. While the presence of NrxIV is well-established in glial SJ, we examined distribution other SJ miR-184 targets within glia and established the presence of kune, Mcr and sin in glial SJs, and M6 and Gli in glial TCJs. miR-184 overexpression in SPG led to decrease in protein localization to the membrane, subsequently leading to defective septate junction structure. Moreover, these larvae exhibited larval lethality and decreased larval locomotion. Interestingly, this loss of miR-184-targetted-SJ proteins leads to mislocalization of other non-miR-184-target SJ proteins like Nervana 2.1. This SJ degradation could be attributed to miR-184 targeting all SJ mRNAs or just the core SJ NrxIV mRNA which might in turn be affecting other SJ protein localization. For this, qRT-PCR will be performed to confirm downregulation of either all SJ mRNA levels or just NrxIV levels upon miR-184 expression. Secondly, we will analyze if this SJ protein defect is rescued by rescuing NrxIV expression in a miR-184-overexpression-background by immunostaining and qRT-PCR analysis. To understand the functional implication of this phenotype, BBB integrity will be assessed by dye penetration assays. Our study will reveal the presence, regulation and interaction of key SJ proteins in SPG crucial for BBB maintenance.

846T **The Influence of Basigin on Focal Adhesion Complexes at the Perineurial Glial Membrane** Sophie F Roth, Vanessa AuldZoology, The University of British Columbia

In *Drosophila melanogaster*, glial cells play an important role in ensheathing and protecting nerves from damage. The perineurial glia are a conserved class of glia that surround perineurial nerves and ensure proper ensheathment through interactions with the extracellular matrix (ECM). Glia-ECM interactions occur through focal adhesion complexes (FACs) comprised of Integrins and intracellular adapter proteins including Talin. Loss of FACs leads to the loss of the perineurial glial sheath and disruption of nervous system function. Integrins also associate with the transmembrane protein Basigin (aka Neuroplastin) in the perineurial glia. Loss of Basigin leads to constriction across the glial membrane leading to deformation of the glial membrane, cytoskeleton and the overlying ECM. Since the loss of Basigin phenotype can be rescued by the loss of Integrin, we hypothesize that Basigin knockdown leads to increased Integrin and FAC activation. Specifically, we hypothesize that Basigin negatively regulates Integrin activation in order to modulate focal adhesion strength across the glial membrane. This leads to the questions: 1. Does the increase in Integrin activation with Basigin knockdown lead to changes in tension across the membrane? 2. What is the role of Basigin in glial FACs?

To investigate these questions, a FRET (fluorescence resonance energy transfer) sensor inserted between the head and rod domain of Talin will be used to visualize and quantify changes in Integrin activation when Basigin is knocked down. The sensor includes a donor fluorophore and an acceptor fluorophore connected by a flexible linker peptide. Since Integrin activation leads to the lengthening of the rod domain of Talin, this will lead to a decrease in FRET as the two fluorophores become separated. FRET acceptor photobleaching will be performed in the peripheral nerves of live larvae to compare controls with Basigin knockdown. As well, an antibody that binds to an epitope of Integrin that is only exposed during activation will be used to assess any change in Integrin activation with Basigin knockdown. This study will help to elucidate the role of Basigin in glial FACs and provide insight into the nature of the interaction between Basigin and Integrin. Overall, investigation into FAC maintenance in glia is an important concept in all animals because of their role in establishing and maintaining glial sheath formation and protecting nervous system function.

847F **Velvet ant venom activates pain-sensing neurons through Pickpocket and Balboa, homologs of DEG/ENaC**

and ASIC channels Lydia J Borjon, Daniel TraceyBiology, Gill Center for Biomolecular Science, Indiana University

The velvet ant *Dasymutilla occidentalis* is called the “Cow Killer” due to its extremely painful sting. In order to test 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. Remarkably, we found that velvet ant venom specifically activates peripheral nociceptors (class IV multi-dendritic neurons), without activating other sensory neurons (such as class III multi-dendritic neurons). We next tested two candidate target channels whose expression is specific to nociceptors: Pickpocket/Balboa and dTRPA1. Pickpocket (Ppk) and Balboa (Bba) are members of the DEG/ENaC and ASIC channel superfamily and have previously been shown to be necessary for mechano-nociception in fly larvae (Zhong et al., 2010). The presence of both Ppk and Bba subunits are required for proper localization to sensory dendrites and the channel subunits likely form a heterotrimer (Mauthner et al., 2014). To test whether Pickpocket is activated by velvet ant venom, we ectopically expressed Ppk and Bba in class III neurons, and this caused these non-nociceptive neurons to be strongly activated by venom. However, expression of Ppk or Bba alone did not cause activation, providing evidence for the first time that Ppk/Bba heteromers form functional channels. On the other hand, expression of dTRPA1, a cation channel sensitive to heat and noxious chemicals, did not render class III neurons sensitive to velvet ant venom. RNAi against ppk or bba in class IV neurons prevented activation by the venom, while the neurons remained sensitive to other noxious stimuli (allyl isothiocyanate). *Dasymutilla occidentalis* venom is composed of 24 small peptides whose amino acid sequences have been determined (Jensen et al. 2021). By screening the synthetic peptides, we have identified noxious components of the velvet ant venom proteome. We have identified at least one of these peptides that is responsible for fast-acting nociceptor activation through Pickpocket channels. Results of ongoing work investigating other venom components, and experiments to determine if this mechanism of action is conserved in homologous mammalian ASIC channels, will be discussed.

848F **Investigating the mechanisms that generate neuronal diversity in the *Drosophila* visual system** Alicia Donoghue, Matthew Bostock, Anadika Prasad, Vilaiwan FernandesUniversity College London

The development of a complex nervous system relies upon precise spatio-temporal patterning to generate diverse cell types in the right place and at the right time. Much work has focused on cell-intrinsic programs that drive cell fate decisions, but extrinsic signals can also impact cell fate decisions. My project is focused on understanding how cellular diversity is generated in the lamina, the ‘simplest’ processing layer of the optic lobe, made up of only five neuron types (L1-L5). During lamina development, post-mitotic lamina precursor cells (LPCs) are arranged into pre-cartridges or lamina units called columns and differentiate in an invariant spatio-temporal pattern, such that the LPC located most distally in a column differentiates as an L2, followed by an L3, L1, L4 and the L5 is located most proximally. Recently our lab showed that post-mitotic LPCs in the youngest columns respond to graded levels of Hedgehog (Hh) along the distal to proximal axis, such that the highest levels of Hh specify the most distal cell types i.e. L2s and L3s, with progressively lower levels specifying more proximal cell types. Therefore, Hh is acts as a morphogen to pattern the lamina, bearing similarities to Shh patterning the vertebrate neural tube. However, in *patched (ptc)* mutant clones, which experience maximum physiological levels of Hh signalling activity, clone cells were a mix of L2 and L3 neuron types, indicating that an additional signal is required to distinguish between these cell fates. Our recent data indicates that Notch signalling may act to further diversify lamina cell identities. I will present data on the impact of disrupting Notch signalling activity in the lamina and my experimental plans to elucidate the molecular mechanisms responsible for notch-dependent lamina neuron specification.

849F **MINIDISCS, a SL7A amino-acid transporter, involved in the amino acid-dependent activity of Kenyon cells, in *Drosophila melanogaster*.**
Julie Delescluse[1], Mégane Simonnet[2], Kevin Piffaretti[3], Yaël Grosjean[1], Gérard Manière[11]Center for Taste and Feeding behaviour, [2]Enalees, [3]Lysarc

Amino acids are small molecules that are essential to living organisms. They are inseparable from cellular metabolism, and they play multiple roles as constituents of proteins, cell signalling cofactors, and also as precursors of hormones and neurotransmitters, and some can act as neurotransmitters. Amino acids are necessary for proper neurological functioning and ensure adapted behaviours. The majority of amino acids come from the diet, and diet modifications could change global free amino acids level within the body, and presumably into the brain.

Neurological diseases such as schizophrenia or autism, present defects in amino acid transport, notably due to a poor regulation of Solute Carrier 7 (SLC7A) family. Five potential SLC7A transporters -MINIDISCS (MND), GENDERBLIND, JhI-21,

SOBREMESA and CG1607- have been identified in Drosophila.

To elucidate the role of SLC7A transporters in the neural circuitry, we focused on the transporter MND a potential ortholog of SLC7A5.

We found that MND is expressed in the adult central nervous system, notably in glia and neurons depending of which *Mnd* regulatory sequence was used. By intersectional genetic strategy, we found that MND is particularly expressed in Kenyon cells forming the α/β and γ lobes of the mushroom bodies (MBs), under the control of one specific regulatory sequence (we called *24-1*). To unravel the role of MND in these neurons, we performed calcium imaging experiments in the MBs with each of the twenty amino acids. Downregulation of MND by RNAi in these neurons impairs calcium activity in the MBs induced by some specific amino acids. We also found that downstream effectors of MND, such as Glutamate Deshydrogenase and Target Of Rapamycin are also involved in this amino acid response.

These results confirm that MND is involved in the amino acid-dependent activity of mushroom bodies. This information opens up a fascinating new field of investigation, which could lead to a better understanding of how nutrients and simple molecules, such as amino acids, can influence neuronal activity and affect behaviour. That could be helpful to bring new clues in the understanding of human neuronal diseases.

850F **Activity-dependent pH transients within the *Drosophila* synaptic cleft enhance synaptic transmission**
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Neurotransmission is highly sensitive to shifts in extracellular pH (pH_e), a phenomenon stemming from the pH_e sensitivity of transmembrane proteins. At the synaptic cleft, synaptic activity itself can drive rapid pH_e transients. Yet, despite the substantial insight gained into short-term forms of synaptic plasticity (STSP) activity-dependent pH_e transients acting on pH sensitive mechanisms during synaptic activity is rarely considered. With powerful genetic tools and a synapse accessible for electrophysiology, Ca^{2+} and pH imaging, the *Drosophila* larval neural muscular junction makes an attractive model for investigating the effects of activity-dependent pH_e transients on STSP and the identity of the pH sensitive transmembrane proteins. Pairing optogenetics with two-electrode voltage clamp we identify the presynaptic rather than postsynaptic compartment to be sensitive to shifts in pH_e imposed by superfusion, where quantal content increases as pH_e rises. The influence of activity-dependent pH_e transients on STSP was examined by increasing the extracellular buffering capacity which led to an increase in frequency depression during burst firing. Enhanced extracellular buffering reduces activity-dependent alkalinization of the cleft, but it is also predicted to partially extinguish acidification in microdomains at sites of synaptic vesicle exocytosis, potentially occluding acid sensing ion channel (ASIC) function. To investigate the potential role of ASIC function in STSP, we inhibited ASIC function with 50uM Benzamil and observed an increase in frequency depression by an amount similar to that observed during cleft buffering. Further, cleft buffering in the presence of Benzamil brought about no additional depression. These data suggest that, at *Drosophila* larval NMJ, brief acid spikes with a progressively alkalinizing cleft effectively recruit and maintain ASIC function, thus enhancing synaptic strength during burst firing.

851F **The Presynaptic Role of Phosphagen Systems** Carlos D Oliva[1], Danielle V Riboul[2,3], Sergio Sempertegui[4], Karlis A Justs[5], Yaouen Fily[1], Gregory T Macleod[1,3,6,71]Wilkes Honors College, Florida Atlantic University, [2]Integrative Biology Graduate Program, CES College of Science, Florida Atlantic University, [3]Jupiter Life Science Initiative, Florida Atlantic University, [4]Department of Physics, Florida Atlantic University, [5]Scripps Research Institute, [6]Institute for Human Health & Disease Intervention, [7]Stiles-Nicholson Brain Institute, Florida Atlantic University

ATP generation via glycolysis and oxidative phosphorylation is too slow to sustain intense neuronal activity and stabilize ATP levels, suggesting there might be another energy pool at play. The phosphagen system, extensively researched in muscles, is an ATP regeneration pathway that quickly produces ATP through ADP re-phosphorylation, catalyzed by a phosphagen kinase. Its presence has been observed in neurons, yet its involvement in neuronal energy metabolism remains poorly understood. To investigate the phosphagen system's impact on ATP levels, we examined *Drosophila* motor neurons in which arginine kinase was knocked down (ArgK1-KD) while using genetically-encoded fluorescent reporters for pH, calcium (Ca^{2+}), and ATP. High frequency stimulation while imaging nerve terminals revealed ATP levels

to fall further in ArgK1-KD terminals. However, cytosolic acidification of neurons is often observed during synaptic activity - capable of quenching fluorescent reporters. Therefore, we obtained recordings of changes in cytosolic pH during activity to correct for the pH-sensitivity of ATP reporters and unexpectedly revealed greater acidification in ArgK1-KD neurons. Correcting for the effect of pH on the ATP recordings did not eliminate the greater fall in ATP levels in ArgK1-KD terminals, leading us to conclude that the increased acidification in ArgK1-KD terminals is caused by either a compensatory increase in glycolysis, or greater activity of the $Ca^{2+}/2H^{+}$ anti-porting plasma membrane calcium ATPase (PMCA), subsequent to Ca^{2+} influx. To investigate whether Ca^{2+} influx was greater in ArgK1-KD terminals, we obtained Ca^{2+} recordings which revealed no greater Ca^{2+} influx in ArgK1-KD terminals, indicating that the greater acidification observed in ArgK1-KD terminals was not due to PMCA activity, but more likely the result of compensatory lactate generation by glycolysis. These data suggests that the phosphagen system's presence in neurons contributes to presynaptic energy metabolism. A better understanding of the role of phosphagen systems in neurons and neuronal bioenergetics in general may provide insight into the causes of various neurodegenerative disorders and potential therapeutic approaches.

852F **5-HT1A regulates axon outgrowth in a subpopulation of *Drosophila melanogaster* serotonergic neurons**
Delaney R Long, Luke Brewer, Dayle Matheny, Breanna Long, Abby Welling, Douglas RoossienBiology, 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. Many behavioral disorders have been correlated with defective serotonergic axon morphologies. 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. We hypothesized that serotonin initiates autoregulation by activating autoreceptor 5-HT1A, thereby inhibiting serotonergic axon outgrowth. To test this, we used primary *Drosophila* serotonergic neurons in which we could manipulate 5-HT1A pharmacologically. We found that both exogenous serotonin and activation of 5-HT1A mimicked autoregulation by reducing axon length and branching, whereas inhibition of 5-HT1A rescued normal outgrowth. We confirmed that 5-HT1A is an autoreceptor in our cultured serotonergic neurons using a genetic labeling approach, and found that 30.7% of serotonergic neurons express 5-HT1A. 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.

853F **Investigating Hippo signaling as a novel regulator of dopamine levels in *Drosophila melanogaster*** Shelley B Gibson[1,2], Samantha L Deal[2,3], Danqing Bei[1,2], Harim Delgado-Seo[2,4], Elaine S Seto[5], Shinya Yamamoto[1,2,3,41]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, [5]Neurology, Texas Children's Hospital

Dysregulation of dopamine (DA) signaling is seen in many disorders including Parkinson's Disease, schizophrenia, and mood disorders among others. Understanding the mechanisms by which DA synthesis is regulated is imperative to develop novel treatments for those affected by dysregulation of DA signaling pathways. 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 presynaptic 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 three kinases of the core Hippo signaling pathway, *hippo*, *mats* and *warts*, as novel regulators of melanin formation in a screen based on darkening of the cuticle with RNAi knockdown (KD). Further investigation confirmed the involvement of *salvador* and *yorkie* in the same process, thus implicating all five key genes of the canonical Hippo signaling pathway as novel regulators of DA. Importantly, I obtained preliminary data that shows KD of *warts* in DArgic cells results in an increase of DA levels in the fly head, indicating that Hippo signaling may regulate DA levels in the nervous system as well. Although the role of Hippo signaling in DA regulation is unknown, YAP (the human homolog for *yorkie*), a transcriptional activator that is negatively regulated by Hippo signaling kinases, has been shown to be involved in DArgic neuron differentiation and survival in mammalian systems. I hypothesize that the Hippo signaling pathway regulates key enzymes involved in DA synthesis such as TH and Ddc, and controls *Drosophila* behavior through alteration of DA signaling in the brain. Future

studies will center on determining the consequences of Hippo signaling modulation on DA levels and synthesis enzymes in the adult *Drosophila* brain, as well as investigation of the role of canonical Hippo signaling in fly behaviors related to DA signaling. These studies will expand our knowledge of Hippo signaling targets in *Drosophila* DArgic neurons, novel regulators of DA synthesis in the *Drosophila* brain, how the Hippo signaling pathway affects DA-related behaviors in *Drosophila* and whether this link is conserved in mammals including human.

854F **A FMRP-Dependent Pathway for the Glial Phagocytosis of Brain Neurons** Rincon Jagarlamudi, Kendal BroadieBiological Sciences, Vanderbilt University

Brain circuit remodeling is a crucial developmental process that is disrupted in a number of different neurological disease states, including Fragile X syndrome (FXS); the leading heritable cause of intellectual disability (ID) and autism spectrum disorder (ASD). Recent work suggests that the developmental clearance of neurons during circuit remodeling requires neuron-to-glia signaling that drives glial phagocytosis. We study these events in the *Drosophila* brain PDF clock circuit, which contains the developmentally transient PDF-Tri neurons. We have previously shown that the Fragile X Mental Retardation Protein (FMRP) is required in neurons to activate and recruit glia for the phagocytosis and developmental clearance of these PDF-Tri neurons.

We have now found that glial Basket (mammalian Jun N-terminal kinase; JNK) signaling and the downstream AP-1 transcriptional pathway are required for the clearance of PDF-Tri neurons. We found FMRP mutants exhibit reduced Basket/JNK translocation into glia nuclei, indicating a disruption in JNK signaling to AP-1 transcriptional control. We discovered this JNK/AP-1 signaling pathway drives the glial expression of Cheerio (mammalian filamin A; FLNA), an F-actin cross-linking protein, which is required for the PDF-Tri neuron clearance. Null *cheerio* mutants and glia-target *cheerio* RNAi similarly prevent removal of the normally developmentally transient PDF-Tri neurons. We also discovered FMRP genetically interacts with Cheerio to impact glia at a cytoarchitectural and functional level. Transheterozygous animals with only one copy of the FMRP and Cheerio genes display an impaired glial actin cytoskeleton and complete blockade of PDF-Tri neuron removal. We discovered that the glial processes in both FMRP and Cheerio mutants have a diminished ability to infiltrate the PDF-Tri region and produce phagocytic markers. We found that neuronal FMRP drives Basket/JNK signaling principally in ensheathing glia, which use the Cheerio/FLNA actin crosslinker to migrate to and clear the PDF-Tri neurons.

Taken together, we conclude that neuronal FMRP drives glial Basket/JNK signaling to induce AP-1 transcription of Cheerio/FLNA to modulate the glial F-actin cytoskeleton to enable glial phagocytosis of the PDF-Tri neurons. Our work identifying structural actin defects in Fragile X and Cheerio mutants contributes to the hypothesis that downstream down-regulation of cheerio in Fragile X animals prevents glial process extension, via their actin cytoskeleton. Future work will also focus on the FMRP-dependent neuron-to-glia signaling mechanism (ligand and receptor) that initiates the JNK/Basket pathway in glia. This clearance pathway has important implications for the study of Fragile X syndrome, as it provides a new cellular mechanism for the disease state and new molecular targets for the development of new therapeutic treatments.

855F **Contribution of Disc Large 1 to AIS protein composition in Drosophila Melanogaster** Nat Casson[1,2], Amelia C Hunter[2], Vanessa Auld[11]Zoology, University of British Columbia, [2]University of British Columbia

The Axon Initial Segment (AIS), which is located adjacent to the axon hillock in a myelinated neuron, is the site of axon potential initiation in vertebrates. This location can be identified by its unique morphology and through increased expression of key AIS components such as scaffolding proteins or ion channels. The structure of the AIS is well characterized in vertebrate myelinating neurons, but it is not well understood in invertebrate and unmyelinated axons. Within Drosophila melanogaster, there is a putative AIS region located at the boundary between the CNS and PNS on the Ventral Nerve Cord of 3rd instar larvae. We identified that the voltage-gated ion channels Shal (potassium channel) and para (sodium channel) are located in this region, as well as scaffolding protein Ankyrin1, in a manner similar to the distribution of proteins at the vertebrate AIS. Furthermore, we found that MAGUK scaffolding Discs large 1 (Dlg1) is concentrated at the AIS. Dlg1 is found colocalized with Basigin (Bsg) and the plasma membrane Ca+2 ATPase (PMCA) suggesting a potential protein complex at the AIS. We found that knockdown of Bsg leads to the loss of PMCA, but PMCA knockdown has no effect on Bsg localization. Dlg1 has multiple protein isoforms, and we found that the L27 and A isoforms are present at the AIS. The effects of loss of Dlg1 on the AIS protein complexes is unknown. We hypothesize that there is a hierarchy of interactions that recruit complex members to the AIS, and that specific isoforms of Dlg1 play a role in complex formation. Using RNAi knockdowns, mutant studies, and proximity ligation assays, this project will determine

which Dlg1 isoforms function at the AIS. We will test how loss of these isoforms alter AIS structures—specifically looking at the localization of Bsg and PMCA—and determine the hierarchy of interactions of these complex members. AIS structures are highly conserved throughout both vertebrate and invertebrate neurons. Consequently, this research will help in our global understanding of the unmyelinated neuronal AIS.

856F **Characterization of phenotypes of piragua (prg) mutant alleles in the nervous system of Drosophila melanogaster** César Cano, Juan R RiesgoUniversidad Nacional Autónoma de México

piragua (prg) encodes a zinc finger (ZF) transcription factor protein belonging to the C2H2 ZF transcription factor family, containing one zinc associated domain (ZAD) and nine C2H2 zinc finger domains. *prg* expression is highly dynamic during embryogenesis (both the transcript and protein) in different epithelial precursors. Two hypomorphic alleles of the gene*, prg1* and *prg3,* are embryonic lethal in homozygosis with a phenocritical period centered during embryogenesis; a small percentage reach the first or second instar. In mutant clones, adult tissues also show aberrant phenotypes. In the imaginal eye mutant ommatidia have dramatic malformations. This suggest that *prg* is required for nervous system development. Here, we study nervous system development requirements in *prg* mutants. We used monoclonal antibodies to mark different neural and glial structures to determine when and where is *prg* needed during embryonic nervous system development and first instar. We used antibodies against ELAV, REPO, 22C10 and BP 102 marking differentiated neurons, glial cells, CNS axons, and PNS axons, respectively. *prg* mutants have defects in both neurons and glial cells, mainly after stage 10 of embryonic development. The main defects are seen in the first three abdominal segments of the Ventral Nerve Cord (VNC), consistent with severe defects seen there in first instar larvae. Brain defects are also seen, even in the initial stages of embryonic development.

857F **Characterizing the *Drosophila* common Dpr/DIP-interacting protein (cDIP) *in vitro* and *in vivo*** Viola I Nawrocka[1], Yeonhee J Park[1], Patryk Polinski[1], Tomasz Slezak[1], Robert A Carrillo[2], Engin Özkan[11]Biochemistry & Molecular Biology, The University of Chicago, [2]Molecular Genetics & Cell Biology, The University of Chicago

Cell surface proteins are involved in virtually all neurodevelopmental processes including axon guidance, dendritic arborization, target selection, and synaptogenesis. Studies of the *Drosophila* extracellular proteome revealed a network of neuronal receptors belonging to the immunoglobulin superfamily of proteins, Dprs (Defective proboscis response) and DIPs (Dpr-Interacting Proteins). The Dpr-DIP interactome was accompanied by a Leucine-Rich Repeat (LRR) protein, common DIP (cDIP), that directly binds to multiple Dprs and DIPs *in vitro* acting as a hub of the network. The last decade uncovered key roles for Dprs and DIPs and their complexes in neuronal wiring of the *Drosophila* nervous system. However, the function of cDIP remains unknown. Here, we sought to understand its interplay with the Dpr-DIP interactome using *in vivo*, biochemical, and biophysical approaches. Staining of fly 3rd instar larvae with soluble cDIP revealed its localization to fly neuromuscular junctions (NMJs), suggesting *in vivo* interactions with Dprs and DIPs that function at the NMJ. To study cDIP›s endogenous expression patterns across fly's developmental stages, we generated synthetic anti-cDIP antibodies. We also present the structure of cDIP in complex with one of the Dprs. The structure in combination with other biophysical approaches suggests that cDIP could interfere with the formation of Dpr-DIP complexes. Our findings suggest a potential regulatory role for cDIP in Dpr-DIP-mediated wiring functions in the nervous system of *Drosophila*.

858F **Expression and functional profiling of sphingolipid enzyme network in the Drosophila nervous system** Chih-Chiang Chan[1], Fei-Yang Tzou[2], Wan-Shyuan Lin[2], Cheng-Li Hung[2], Yi Hsiao[2], Hsin-Chun Yeh[2], Kai-Hung Chen[2], Chia-Heng Hsu[21]Physiology, National Taiwan University, [2]National Taiwan University

Sphingolipids are a diverse group of lipids serving as both structural components and signaling molecules. Their synthesis and degradation are regulated by a set of sphingolipid-metabolizing enzymes, mutations of which lead to various types of neurological disorders. To systemically reveal how sphingolipid homeostasis is maintained, we have generated a complete set of reporter flies to visualize the entire network of sphingolipid metabolizing enzymes. The reporter flies were CRISPR-engineered to knock-in HA-T2A-Gal4 cassette at the c terminus of the coding region in the endogenous locus, allowing for expression profiling at both the transcription and translation levels. Using this toolset, we constitute a 4D map of the enzymatic network, showing the spatiotemporal patterns of the expressing cells and functioning cells of each enzyme in the larval and adult CNS, as well as the leg as a peripheral tissue, in the contexts of normal aging, metabolic challenge, and neurodegenerative diseases. Our preliminary results identify distinct cell types for ceramide synthesis and degradation and highlight the glial role in degrading complex sphingolipids. Our data also suggest an expansion of several key metabolizing proteins from its expressing cells, underscoring the importance of neuro-glia

communication. The protein distribution of the key enzymes are altered upon neurotoxic challenges, suggesting a regulatory role of these enzymes. From the comprehensive 4D map, we argue that there is not an omnipotent cell in the brain that does everything to cell-autonomously regulate sphingolipid homeostasis.

859F **Two distinct mechanisms of Plexin A function in optic lobe development** Maria Bustillo[1], Jessica Douthit[2], Jessica E Treisman[31]Cell Biology, NYU School of Medicine, [2]University of California, San Francisco, [3]Cell Biology, New York Univ Med Ctr

The development of neural circuits requires neuronal processes to select the appropriate synaptic partners from many different choices within a complex environment. This selection process can be simplified by segregating different types of connections into distinct regions or layers. In the neuropil of the medulla, the largest of the *Drosophila* optic lobes, around 100 different types of neurons form synaptic connections in 10 characteristic layers. We have found that the cell surface protein Plexin A (PlexA) is required for this layered organization to develop correctly. In *plexA* mutants, neuropil layers labeled by several synaptic markers become wider and less distinct than in controls, and the arborizations of individual cell types also extend over a wider area. PlexA protein is enriched in tangential neuron projections in the M7 layer, and removing *plexA* from the precursors of tangential neurons results in layering defects. PlexA is capable of interacting with three members of the Semaphorin family. We find that *semaphorin 1A* (*sema1A*) mutants have similarly disrupted medulla layering, while the medulla develops normally in *sema1b* and *sema5c* mutants. PlexA was originally described as a receptor for Semaphorins, but in some contexts it is thought to act as a ligand instead. We show that deleting the cytoplasmic domain of endogenous PlexA by CRISPR does not result in the medulla layering defects seen in *plexA* null mutants, suggesting that layer formation does not require the receptor function of PlexA. In contrast, a change in the overall shape of the medulla neuropil is observed both in null *plexA* mutants and in mutants lacking the cytoplasmic domain. Therefore, PlexA appears to act as a receptor to constrain the dimensions within which neurites form synaptic connections in the medulla, but as a ligand for Sema1a to establish the coordinates of specific synaptic layers.

860F **Organizational control of olfactory neural circuit architecture by Fat2, an atypical cadherin** Khanh M. Vien, Qichen Duan, Pelin VolkanDuke University

Appropriate neural circuit architecture is critical for the correct transfer of information from peripheral sensory neurons to higher processing units in the central brain. In the D. Melanogaster olfactory system, 50 olfactory receptor neuron (ORN) classes are defined by their expression of, generally, a single and unique olfactory receptor gene and the class-specific discrete synaptic field within the antennal lobes. During development, each ORN class must extend their axons long distances into the central brain to converge onto a discrete, class-specific target region, called glomeruli, where they synapse with projection neurons (PNs) and local interneurons (LNs). A large volume of work in the past two decades investigating the role of cell surface molecules revealed that cell-cell adhesion drives many neurodevelopmental processes from axon guidance and target selection to synaptogenesis. Indeed, transcriptome studies from our lab, as well as others, show that axons of the same ORN class express a signature combination of cell surface molecules that promote same-class recognition when surrounded by a diverse population of ORNs. Identifying how the function of each cell surface protein contributes to glomerular organization requires genetic and neuroanatomical studies. In an RNAi screen for mutations in cell surface proteins that led to the disruption of the glomerular architecture of 4 ORN classes, we identified mutations in the atypical cadherin fat2, also known as kugelei. kugelei mutants show major deformities in glomerular morphology of glomeruli targeted by Or47b, Or23a and Or47a ORNs. Morphology of glomerulus targeted by Gr21a neurons was not affected. We validated the screen results by further characterizing the glomerular phenotype in other RNAi knock down and CRISPR generated fat2 null mutants. Additionally, we found that fat2 mutants with a disrupted intracellular domain, but intact extracellular domain, phenocopy null mutants. Our expression profile analysis shows fat2 is widely expressed throughout the peripheral (antenna) and central olfactory system (antennal lobe). Using PN and LN drivers to colabel these non-ORN neurons in conjunction with fat2 positive cells, we found that PNs express very low levels of Fat2 and a subpopulation of LNs labeled by 499-GAL4 highly expressed Fat2. To clarify whether LN expression of Fat2 was necessary for glomerular development, we performed an LN specific knockdown of Fat2 using 499-GAL4 driven fat2 RNAi and found no difference between the control and the knockdown groups. These results suggest that cell adhesion induced intracellular signaling downstream of the Fat2 protein within ORNs contribute to glomerular architecture and organization in the olfactory system.

861F **Deciphering the molecular clock controlling the neurogenesis diversity in drosophila's medulla** Khaled Ben El Kadhi[1], Claude Desplan[21]CGSB, New York University Abu Dhabi, [2]Department of Biology, New York University

The Drosophila compound eye is composed of 800 unit eyes; each contains 8 photoreceptors (PRs). The visual information collected by the PRs is transferred to the 4 visual processing centers of the optic lobe, the lamina, medulla, lobula and lobula plate. The medulla is the most complex structure of the optical lobe, it consists of 40,000 neurons. These neurons are the progeny of 800 medulla NeuroBlasts (NBs) that derive from a larval neuroepithelium, the Outer Proliferation Center (OPC). The OPC's NBs divide asymmetrically to self-renew and to produce a Ganglion Mother Cell (GMC) that will produce two different medulla neurons. It was shown that the sequential expression of 6 temporal transcription factors (tTF) (Hth-Klu-Ey-Slp-D-Tll) in NBs generates neuronal diversity. Although the tTF cascade was identified, we do not have dynamic information about the timing mechanisms, the duration of each temporal identity and how the transition occurs between tTFs.

The general aim of my work is to develop a NBs primary culture to define the molecular clock of the tTF cascade using live-imaging (L-I).

We used the CRISPR-Cas9 system to endogenously tag the tTFs with different fluorescent proteins (Hth::GFP Klu::RFP Ey::BFP...) and/or transcriptional reporters MS2 or PP7. Using L-I we quantified the duration of multiple competence windows, the number of cell divisions as well as the duration of the transitions. To test if the molecular clock of the tTFs cascade is intrinsic to the NB. we set a quasi-isolated NB culture (qiNBc). We found that the transition between tTFs is maintained in qiNBc, suggesting that the transition signal is intrinsic to NB or comes from its progeny. We tested this hypothesis by selectively destructing the GMC or neurons (N) using Laser microablation techniques.

These dynamic data will allow us to decipher the molecular clock of tTF and provide essential information about the mechanisms responsible for the neuronal diversity in the Drosophila optic lobe, which will likely also apply to temporal patterning observed in mammals. More importantly, this will also enable us to understand how to program a naive neural stem cell to produce a specific type of neuron that could be used for cell replacement therapy.

862F **Persistence of courtship behavior neurons from larval to adult life in *Drosophila*** Julia Diamandi, Julia Duckhorn, Kara Miller, Sofia Leone, Troy ShirangiVillanova University

The neural circuits for courtship behavior in *Drosophila* are understood to arise largely from postembryonic neurons, dedicated to adult-specific behaviors. The extent to which these circuits also consist of embryonic-born neurons with former activities in larval life is unknown, however. The *dissatisfaction* gene (*dsf*) in *Drosophila* encodes a *tailless*-like orphan nuclear receptor that influences the development of courtship behaviors in both sexes. We recently identified a small sexually dimorphic population of *dsf*-expressing interneurons in the adult abdominal ganglion—the DDAG neurons—whose activity is necessary and sufficient for vaginal plate opening behavior in virgin females and abdominal bending in males during courtship. Here, we demonstrate that the DDAG neurons consist of at least five anatomical subtypes and identify subtypes that regulate the opening of the vaginal plates. We provide evidence that the DDAG neurons exist in the abdominal ganglion of larvae as mature, sexually monomorphic neurons that are born during embryogenesis. During metamorphosis, the DDAG neurons gain expression of the sexual differentiation gene, *doublesex*, and become repurposed for sexually dimorphic functions in adult courtship. Our results suggest that the neural circuits for courtship behaviors arise in part from sexually monomorphic larval neurons that are reallocated during pupal life for sexually dimorphic functions in the adult.

863F **A novel transmembrane protein plays a role in photoreceptor morphogenesis** Fareeha Syeda, Khanh Lam-Kamath, Deepshe Dewett, Rory Delaney, Jens RisterUniversity of Massachusetts, Boston

Chronic vitamin A deprivation (VAD) in humans results in photoreceptor death and is the leading cause of preventable childhood blindness. Like humans, Drosophila photoreceptors are severely damaged upon VAD, but VAD does not cause photoreceptor death in flies. Previously, our lab identified a novel transmembrane protein, Mps, that stabilizes photoreceptors that are damaged by VAD. Here, we investigate another role of Mps during the development of the light-sensing compartments (rhabdomeres) of the photoreceptors.

We found that Mps is expressed in the terminally differentiating pupal rhabdomeres beginning from the 50% to 93% pupal development. Moreover, we discovered that at 75% pupal development, mps mutant rhabdomeres were smaller and thinner compared to wildtype ones, indicating that Mps is required for rhabdomere development. To gain insights into the underlying mechanism, we investigated the impact of the mutation of mps on two major structural proteins, the scaffolding protein InaD and the unconventional myosin NinaC. Strikingly, we discovered that Mps is required for the rhabdomeric localization of InaD and NinaC. Conversely, Mps was not present in the outer photoreceptors rhabdomeres

of inaD mutant retinas, but only in the rhabdomere of the inner photoreceptor.

Taken together, our findings demonstrate the role Mps plays in development by interacting with other proteins. Our future studies will aim to further characterize the interaction and co-dependence of these proteins by analyzing Mps expression and localization in ninaC mutant retinas at various pupal stages as well as in adults. Ultimately, understanding the function of the novel membrane protein Mps in stabilizing damaged photoreceptors could inspire therapies for human eye diseases.

864F **Analysis of the Guanine Nucleotide Exchange Factor, *GEFmeso*, in the Drosophila Neuromuscular Junction** DAVID J OLSON, Aliyah Sander, Ben KirkhamBiology, UW-Platteville Baraboo Sauk County

Small Rho Family GTPases act as molecular switches that mediate signaling events at the cell surface with downstream cellular responses. These protein switches are activated by Guanine Nucleotide Exchange Factors (GEFs) and deactivated by GTPase Activating Proteins (GAPs). This study examines the role of the Drosophila gene, *GEFmeso* in the development of the neuromuscular junction. GEFmeso has been previously shown to regulate the Rho Family GTPases Rala and Cdc42 in specifying the Drosophila leg and wing morphology. GEFmeso is thought to function with a GAP partner, called Crossveinless-C (CV-C) in wing development. Recent work has described a role for CV-C in regulating the neuromuscular retrograde signal mediated Glass Bottom Boat (GBB). Loss of function *cv-c* mutations cause an increase in neuronal T-bars but have no effect on bouton number. Here, we extend this analysis to *GEFmeso* loss and gain of function mutations. We find that GEFmeso appears to be necessary and sufficient for proper neuromuscular junction in the developing larva.

865S **Cytodomain-independent guidance of longitudinal axons by *Drosophila* Robo3** Jessie Agcaoili, Timothy A. EvansUniversity of Arkansas

Roundabout (Robo) family transmembrane proteins regulate a number of axon guidance outcomes in bilaterian animals, including their evolutionarily conserved role in Slit-dependent midline repulsion. In *Drosophila*, three Robo family members (Robo1, Robo2, and Robo3) each have specialized roles in regulating midline crossing and the formation of longitudinal axon pathways in the embryonic ventral nerve cord.

Drosophila Robo3 is required for the formation of longitudinal axon pathways in specific medial-lateral positions in the ventral nerve cord. In the absence of *robo3*, axons that normally form pathways in the intermediate region instead join medial pathways closer to the midline. Although Robo3 has been hypothesized to act as a canonical Slit receptor to position longitudinal axons by signaling axon repulsion in response to a midline-derived Slit gradient, this model has not been directly tested.

To gain insight into the mechanism by which Robo3 directs longitudinal pathway formation, we used CRISPR/Cas9-based gene modification to create a version of the *robo3* gene encoding a truncated Robo3 protein lacking its cytoplasmic domain (Robo3ΔC). We show that Robo3ΔC protein expressed from this modified locus is stably expressed and localized to neuronal axons in the embryonic ventral nerve cord, but that aspects of Robo3ΔC localization differ from that of full-length Robo3. We also show that Robo3-dependent guidance of longitudinal axons in the embryonic ventral nerve cord is partially compromised in $robo3^{robo3\Delta C}$ embryos, but intermediate FasII-positive axon pathways still form correctly in some segments in these modified embryos. Our results indicate that *Drosophila* Robo3 guides embryonic longitudinal axons via a mechanism that is at least partially independent of its cytoplasmic domain.

866S **Are the functions of Netrin and Frazzled to guide axons conserved among insects?** Piyasi Ghosh, Tim EvansUniversity of Arkansas

The Netrin-Frazzled/DCC signaling pathway promotes midling crossing of axons in the nervous system of insects and other bilaterian animals. Netrin-Frazzled is the main midline attractive pathway that guides axons to cross the midline in *Drosophila*. Orthologs of the pathway ligands (Netrins) and receptors (known as Frazzled [Fra] in insects and Deleted in Colorectal cancer [DCC] in vertebrates) are widely conserved in bilaterians. However, the regulatory roles of the above-mentioned in insects other than *Drosophila* are not yet well understood. This project aims to compare the midline attractive roles of the Frazzled receptor in the flour beetle (*Tribolium castaneum*) and fruit fly (*Drosophila melanogaster*) using CRISPR/Cas9-mediated gene replacement. The research approach includes CRISPR modifications to replace the *Drosophila frazzled* gene with HA-tagged cDNAs encoding *Drosophila* Frazzled (DmFra) or *Tribolium* Frazzled (TcFra). We compare the expression of DmFra and TcFra in the *Drosophila* embryonic CNS, and examine midline crossing of axons to

see if TcFra is able to rescue DmFra's role in promoting midline crossing of axons in *Drosophila*. Our project aims to gain insight into the evolutionary conservation of axon guidance in insects

867S **Structure-function studies of Drosophila Robo3 immunoglobulin domains using CRISPR gene replacement** Ayawovi Selom Ametepe, Tim EvansUniversity of Arkansas

Neurons extend membrane processes called axons during development of the nervous system. Each axon is guided through the developing embryos by extracellular cues, which are often secreted or membrane-associated proteins produced by cells. The axon is capable to sense these 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. Drosophila Robo3 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 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 then collected embryos carrying the modified robo3 alleles. Embryos were stained with antibodies to label the longitudinal axon pathways. We compared these embryos with wild type and robo3 mutant embryos to determine whether the different modified alleles can substitute for Robo3 to promote longitudinal pathway formation.

868S **A critical DIP-α/dpr10 affinity window is required for proper leg motor neuron arborization** Davys Lopez[1,2], Shuwa Xu[3], Alina Sergeeva[4], Phinikoula Katsamba[2], Barry Honig[2,4,5,6], Lawrence Shapiro[2,5], Kai Zinn[3], Richard Mann[2,4,51]Department of Genetics and Development, Columbia University Medical Center, [2]Zuckerman Mind Brain and Behavior Institute, Columbia University, [3]Division of Biology and Biological Engineering, California Institute of Technology, [4]Department of Systems Biology, Columbia University Medical Center, [5]Department of Biochemistry and Molecular Biophysics, Columbia University, [6]Department of Medicine, Columbia University

For flies to walk properly, motor neurons (MNs) from the ventral nerve cord need to extend ipsilaterally, reach the correct muscle, and arborize appropriately during development. The canonical view of how this is achieved is that cell surface molecules are expressed pre- and post-synaptically that bind to each other like molecular "lock-and-keys" that guide neurons to their targets. The binding affinities of these molecules can vary by a range by more than 100-fold. In the fly leg neuromuscular system, a small handful of MNs express *DIP-α* and their target muscles express its cognate partner, *dpr10.* Although, both of these molecules are necessary for the maintenance of MN-muscle contacts, the role that specific affinities play in this process has not been examined. Here we knocked-in mutations into *DIP-α* and *dpr10* that either decrease or increase the affinity between these two proteins. We find that compared to control animals, decreasing the affinity results in phenotypes similar to *DIP-α* or *dpr10* null animals, where MN axons fail to maintain contacts with their muscle targets and retract their filopodia, resulting in stunted and branchless axons. Surprisingly, we find that if the affinity increases past a certain threshold, a similar branchless phenotype is observed in adult legs. Live imaging during pupal development shows that MN filopodia are unable to productively engage their muscle targets**.** Based on these observations, we propose that DIP-*α*::Dpr10 bind in *cis* in MNs, as well in *trans* (between MNs and muscles)*,* and when the affinity is too high, the interaction in *cis* outcompetes the trans interaction. Further support for this model comes from experiments in which altering the ratio of DIP-*α*::Dpr10 in MNs can also lead to branchless phenotypes.

869S **Developmental activity is shaped by neuropeptide signaling** Sassan Suarez[1], Jun Reichl[1], Harpreet Randhawa[2], Orkun Akin[11]Neurobiology, UCLA, [2]UCLA

Stimulus-independent neuronal activity is important for the organization and refinement of neural circuits in the

developing mammalian brain. Recently, comparable activity was described in both embryonic and pupal stages of nervous system development in *Drosophila*. Despite its apparent ubiquity in developing brains, the mechanisms that initiate and shape stimulus-independent activity are not well understood. In the fly pupa, patterned, stimulus-independent activity (PSINA) engages the whole brain in globally coordinated, stereotyped cycles of active and silent phases during the last two days of metamorphosis. The aim of this work is to find the molecular and cellular components which initiate PSINA and establish its characteristic patterns. Recently, we reported that a population of 2,000 neurons within the expression domain of the cation channel *Trpγ* is critical to PSINA. Loss-of-function and neuronal silencing studies indicated that this population contains a PSINA relay network operating under a presumptive pacemaker or central pattern generator circuit. As many such circuits are modulated by neuropeptides, we undertook a neuronal silencing-based screen through neuropeptide expression domains in search of signaling modules which regulate PSINA. We found that manipulating neuronal activity in the expression domains of the neuropeptides ITP (Ion Transport Peptide), nplp1 (Neuropeptide-like Precursor 1), Proc (Proctolin), and sNPF (Short Neuropeptide F) and their known or putative receptors significantly alter PSINA. Specifically, additional characterization through neuronal activation and loss-of-function analyses is revealing that different neuronal populations have distinct roles in establishing the amplitude, cycle period, and active phase structure of PSINA. Moving forward, we aim to identify the 'PSINA-active' subpopulations of each expression domain and study how they work in concert to initiate and sculpt the characteristic patterns of activity. These observations lend support to the notion that PSINA, and perhaps developmental activity more broadly, is a genetically programmed, as opposed to an emergent, feature of nervous system development.

870S **A non-neural miRNA cluster mediates hearing via two neural targets** Binglong Zhang, Binglong Zhang, Eric LaiDevelopment biology, MSKCC

In contrast to the first identified nematode miRNAs, which were identified on the basis of their strong cell lineage defects, the vast majority of miRNA knockouts across diverse species lack overt and penetrant developmental abnormalities. Here, we show that the mir-279/996 locus is absolutely essential for development of Johnston's Organ (JO), the primary proprioceptive and auditory center in Drosophila antenna and equivalent of the mammalian inner ear. Deletion of this miRNA locus results in highly aberrant cell fates in JO including loss of scolopale cells and ectopic neurons, and mutant animals are completely deaf in electrophysiological assays. Although miRNAs typically have numerous conserved targets, genetic interaction tests show that double heterozygosity for two neural targets (elav and insensible) can fully rescue development and function of JO. This work uncovers critical post-transcriptional regulation of specific genes that governs cell fate specification in the auditory system.

871S **The axonal localization of Dual Leucine Zipper Kinase is essential for its protein turnover by Highwire** Seungmi Kim, Monika HK Singh, Jung Hwan KimBiology, University of Nevada, Reno

Proper protein localization is essential for many biological functions. Dual Leucine Zipper Kinase (DLK) is an axonal protein that mediates multiple stress signals in axon development, regeneration, and neuronal cell death. However, the molecular mechanism underlying the axonal localization of DLK is not known. In this study, we found that Wallenda (Wnd), a *Drosophila* ortholog of DLK, is highly enriched in the axon terminals of *Drosophila* sensory neurons and that the palmitoylation site at amino acid (aa) -130 of Wnd is essential for its axonal localization. We found that Wnd colocalizes with the *Drosophila* Huntingtin-interacting protein 14 (dHIP14), a protein palmitoyltransferase in the somatic Golgi complex. The axonal localization of Wnd was significantly reduced by *dHIP14* mutations. These suggest that Wnd is palmitoylated by dHIP14 on the somatic Golgi complex for axonal targeting. As a critical kinase in stress signaling, Wnd protein levels are under strict control by Highwire (Hiw), an evolutionarily conserved E3 ubiquitin ligase. We found that Wnd down-regulation by Hiw occurs in the axon terminals, but not in the cell body. These results suggest that DLK is actively transported out of the neuronal cell body for Hiw-mediated suppression in the axon terminals. Our findings uncover novel mechanisms underlying subcellular localization of Wnd and provide insights into how axonal transport is coupled to regulated protein turnover.

872S **Mob4's phospho-binding motif is essential for viability and neuronal function** Amanda L. Neisch, Thomas S. HaysGenetics, Cell Biology, and Development, University of Minnesota

Mob4 is a gene whose expression is significantly downregulated in Alzheimer's disease, but whose function is yet to be determined. Mob4 is a core component of the STRIPAK complex, a highly conserved, multi-protein complex that contains kinases and the phosphatase PP2A. We have previously demonstrated that STRIPAK complex proteins, including

Mob4, are required for axonal transport of cellular cargos. However, the molecular function of Mob4 within the STRIPAK complex is not understood. Mob4 contains a conserved phospho-binding motif that in other Mob family proteins binds phosphorylated kinases. To determine if this phospho-binding motif was required for Mob4 function in neurons we used depletion and rescue assays. For the rescue assays we engineered fly strains to express Flag-tagged Mob4 transgenes, both wild type and Mob4 in which the phospho-binding pocket is mutated. Depletion of Mob4 specifically in neurons results in animals that exhibit an unexpanded wing phenotype, similar to mutants that lack the neurohormone bursicon, which is packaged into dense-core vesicles for transport and secretion. These animals also display a climbing defect, suggesting motoneuron dysfunction. Further, we show defects in dense core vesicle transport when Mob4 is depleted. All of these phenotypes are rescuable by expression of wild type Mob4, but not by a phospho-binding defective Mob4, demonstrating that the phospho-binding motif is crucial for neuronal function. Mob4 is required for animal viability. To further determine if the phospho-binding activity of Mob4 was crucial for viability we mutated the phospho-binding motif within the endogenous Mob4 gene using CRISPR technology. These Mob4 phospho-binding mutant animals do not survive to adulthood, dying at larval or pupal stages, similar to Mob4 null mutants, further demonstrating that the phospho-binding motif of Mob4 is essential for its function. We propose that Mob4 functions, through its phospho-binding motif, to recruit a kinase into the STRIPAK complex for PP2A-mediated dephosphorylation and a reduction in kinase activity.

873S **Flies with altered developmental neural activity have sleep deficits as adults** Jun Reichl[1], Pranav Kadiyala[2], Orkun Akin[11]Neurobiology, UCLA, [2]UCLA

In the developing brain, spontaneous activity is important for neural circuit maturation. To date, little is known about the relevance of such developmental activity to adult behavior and neuropathology. Here, we follow up on the recent discovery of patterned, stimulus-independent, neural activity (PSINA) in the developing *Drosophila* brain. PSINA engages the whole brain in a coordinated and highly structured fashion during the second half of pupal development. A small population of some 2,000 neurons expressing the cation channel *Trpγ* are critical to PSINA: Attenuating activity in this population, either with the *trpγ* null mutation or neuronal silencing approaches, leads to significant changes in brain-wide PSINA and altered synaptic development. In this study, we use this genetic handle on PSINA to ask if altered developmental activity affects sleep. We found that *trpγ* nulls have sex-specific defects in sleep dynamics: Both sexes show impaired sleep consolidation while males also exhibit decreased nighttime sleep. To ask if these phenotypes are due to disrupted PSINA or to a concurrent requirement for *Trpγ* function in the adult, we are carrying out timed-rescue experiments. A developmental origin for sleep deficits would indicate a role for PSINA in the maturation of sleep circuitry, whereas a concurrent requirement would implicate the *Trpγ*-expressing neurons in the maintenance of wild-type sleep in the adult. Notably, *trpγ* mutants were shown to have learning deficits in courtship conditioning, a behavioral paradigm which requires sleep for memory consolidation. Together, these observations raise the possibility that brain-wide developmental activity is necessary for the refinement or correct function of the circuitry of sleep, which itself is critical to learning, memory, and nervous system health.

874S **Clock protein-chromatin complexes are assembled within nuclear condensates to enable circadian gene repression** Dunham Clark[1], Ye Yuan[2], Qianqian Chen[2], Christopher Wilson[2], Swathi Yadlapalli[21]Cell & Developmental Biology, The University of Michigan, [2]Department of Cell and Developmental Biology, The University of Michigan

Circadian clocks are based on negative transcription translational feedback loops. We have recently demonstrated that Drosophila core clock proteins, PERIOD and CLOCK, are organized into a few dynamic nuclear condensates in clock neurons specifically during the repression phase (Xiao et al., PNAS 2021). However, what role do clock protein condensates play in gene repression remains to be determined. To address this question, it is critically important to identify the components of clock protein condensates. Here, using CRISPR generated knock-in flies we show that other key clock proteins, TIMELESS and CRYPTOCHROME, are colocalized to the PERIOD condensates during the repression phase. We found that TIMELESS and CRYPTOCHROME nuclear entry occurs several hours after PERIOD nuclear entry, and their nuclear entry is abolished in per01 null mutants. Next, we developed a method to perform simultaneous clock protein-gene imaging by combining PER protein imaging and HCR-FISH (Hybridization chain reaction RNA-FISH)27, in which we used probes targeting an intron of timeless to visualize timeless pre-mRNA (which corresponds to timeless gene location) via a nuclear signal. Using this method, strikingly, we found that timeless gene is colocalized to or located in close vicinity of one of the PERIOD condensates specifically during the repression phase. Together, these studies demonstrate that the clock machinery consisting of core clock proteins and genes is assembled within a few nuclear condensates during the repression phase, providing important, new insights into the biological role of clock protein condensates.

875S **Regulation of neuronal development and function by the eukaryotic protein translation initiation complex** Erik Nolan[1], Iris Chin[2], Cassie Vernier[3], Yehuda Ben-Shahar[11]Biology, Washington University in St. Louis, [2]University of California San Francisco, [3]Biology, University of Illinois Urbana-Champaign

The neuronal proteome is shaped by precise spatial and temporal regulation of translation. Disruptions in the regulation of neuronal protein synthesis are thought to explain some of the cognitive and behavioral pathologies associated with neurodevelopmental disorders such as autism spectrum disorder and Fragile X syndrome. The *eukaryotic translation initiation factor 4H* (*eIF4H*) gene is a member of the highly conserved eIF4F translation initiation complex that is responsible for initiating most 5' m^7G cap-dependent translation. In humans, *eIF4H* resides in the genomic region (7q11.23) that is deleted in Williams syndrome (WS), a rare multi-system disorder characterized by sociocognitive deficits such as hypersociality. However, how *eIF4H* deletion might be playing a role in the etiology of WS remains unknown. We found that in *Drosophila,* neuronal knockdown of *eIF4H1* results in abnormal fly social behavior as well as aberrant morphology of larval multidendritic (md) sensory neurons. Therefore, we hypothesize that *eIF4H*-dependent regulation of the neuronal proteome plays an important role in higher brain functions, such as those disrupted in WS and related syndromes. We are testing this hypothesis by leveraging cell-specific genetic approaches to characterize the specific role of *eIF4H1* in shaping the neuronal proteome, and its subsequent impact on neuronal morphology and behavior. In doing so, we hope not only to model the neuronal mechanisms that underlie the unique sociocognitive profile of WS patients, but also uncover mechanistic principles by which translation initiation impacts neuronal functions and behavior.

876S **Synaptotagmin β regulates neuropeptide release and circadian output in *Drosophila*** Hsueh-Ling Chen, Yang Chen, Aidan Dermady, Jingce Lei, Jun Yin, Quan YuanNational Institute of Neurological Disorders and Stroke

Neuropeptide signaling plays an important role in regulating a myriad of developmental, physiological, and behavioral functions throughout an animal's life cycle. Although much progress has been made in identifying the functional roles of a variety of neuropeptides, the molecular mechanisms controlling neuropeptide release remain largely elusive. The *Drosophila* lateral ventral neurons (LNvs) serve as a suitable system to study this fundamental question. LNvs produce pigment-dispersing factor (PDF), a neuropeptide well-known for its function in synchronizing of the central clock system and regulating the circadian output, such as the locomotor activity and sleep. Notably, despite the high level of *pdf* transcripts produced throughout the day, the amount of PDF peptides at the axonal terminal region of LNvs shows clear circadian oscillations, suggesting a mechanism that tightly controls the trafficking and release of PDF. Using cell-type-specific transcriptome analyses and genetic studies, we first examined a group of LNv enriched genes associated with vesicle trafficking and release. Our behavioral and anatomical data suggest that the *Drosophila* Synaptotagmin β (Sytβ), an atypical synaptotagmin enriched in peptidergic neurons, acts as a part of the control mechanism that regulates PDF release in LNvs. Similar as the *pdf* null mutants, *sytβ* knock out flies show specific circadian behavior deficits, as well as abnormal distribution of PDF peptides. In addition, using the transgenic expression of chimeric proteins of syt1 and sytβ, we demonstrate that the C2B domain of sytβ mediates its inhibitory activity on PDF release and provide evidence for a role of disinhibition in promoting neuropeptide release. Taken together, our genetic studies in the *Drosophila* system help us identify an inhibitory synaptotagmin that specifically regulates neuropeptide release. This finding is potentially generalizable to other circuits and organisms and could help improve our understanding of the fundamental mechanisms underlying neuromodulation of behaviors and brain states.

877S **Identifying Critical Mechanisms of Dense Core Vesicle Sorting, Trafficking and Fusion in *Drosophila*** Kiel OrmerodBiology, MTSU

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* in order to fluorescently tag 9 different DCV proteins to address fundamental questions about DCV biology. We have identified several critical resident DCV proteins that are 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 these tagged versions of DCV cargo we are beginning to characterize cellular mechanisms

of sorting of different uniquely tagged 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 new tools developed here enable a more comprehensive understanding of the critical mechanisms of DCV sorting, trafficking and secretion *in vivo*.

878S **Neuronal excitability modulates developmental time in *Drosophila melanogaster*** Daniel Ruiz, Alejandra Chavez, Alexis HillCollege of the Holy Cross

Developmental time is an essential trait that affects the reproductive success of animals, and is known to be regulated by many genes and environmental conditions. Despite this, an understanding of mechanisms by which cellular processes influence the developmental timing of an organism is lacking. The nervous system is known to control key processes that affect developmental time, including the release of hormones that signal transitions between developmental stages. Here we show that the excitability of neurons plays a crucial role in modulating developmental time. Genetic manipulation of neuronal excitability through the voltage-gated potassium channel *seizure (sei)* and the bacterial voltage-gated sodium channel *NaChBac* in *Drosophila melanogaster* bidirectionally alters developmental time. Developmental time is faster in animals with increased neuronal excitability, and slower in animals with decreased neuronal excitability. The effect of neuronal excitability on developmental time is seen during embryogenesis and later developmental stages. We also find that *sei* mutants exhibit more and earlier movement during embryogenesis. Furthermore, we show that the faster developmental time observed in *sei* mutants with increased excitability is not accompanied by decreased bodyweight or longevity, detrimental phenotypes that sometimes accompany faster development. Observed phenotypic plasticity in the effect of genetically increasing neuronal excitability at different temperatures, a condition also known to modulate excitability, suggests there may be an optimal level of neuronal excitability, in terms of shortening developmental time. We find that selectively modulating the excitability of peptidergic neurons is sufficient to alter developmental time, suggesting the hypothesis that the impact of neuronal excitability on developmental time may be at least partially mediated by peptidergic regulation of hormone release. Together, our data highlight a novel connection between neuronal excitability and developmental time, with broad implications related to organismal physiology and evolution.

879S **Astrocyte regulation of excitatory synapse formation** Sarah D Ackerman[1], Hallie Youker[2,3], Katie Schneider[4], Nelson A Perez-Catalan[5], Sonja A Zolnoski[61]Pathology and Immunology, Washington University School of Medicine, [2]Washington Univeristy School of Medicine, [3]Saint Louis University, [4]Pathology and Immunology, Washington Univeristy School of Medicine, [5]Albert Einstein College of Medicine, [6]University of Pennsylvania

Complex neuronal networks are assembled through the birth of immature synapses between axons and dendrites, followed by synapse maturation and refinement. This three-step process relies heavily on molecular mechanisms from glia to establish functional networks. A subset of glial cells, known as astrocytes, can modify synapse function by secreting extracellular signals in an activity-dependent manner. Astrocytes have also been shown to directly instruct or inhibit the formation of excitatory glutamatergic synapses. *In vitro* approaches have identified many astrocyte-secreted molecules that modify glutamatergic synapse dynamics. Presently, little is known of astrocyte-synapse dynamics *in vivo*, nor the mechanisms by which astrocytes regulate the formation of other types of synapses. We used *Drosophila* to uncover astrocyte-derived factors involved in formation of excitatory cholinergic synapses. We conducted a reverse genetic RNAi screen using the astrocyte-specific *alrm-Gal4* to knock down genes in astrocytes. Concurrently, using Flippase tools (FLP), we labeled both neuronal membranes and their pre-synaptic sites (Brp+) using Synaptic Tagging with Recombination (STaR) to assess non-cell autonomous changes in synapse number. We performed two parallel screens, the first labeled individual dorsal bipolar dendritic (Dbd) sensory neurons that form cholinergic synapses. The second labeled Kenyon cells, which generate cholinergic synapses localized in the mushroom body. Excitingly, we have determined that the major astrocyte-secreted molecules that induce synapse formation (e.g. TGF-β) or inhibit synapse development (*e.g.* SPARC) in vertebrates are conserved in fly. More insights on the effects of novel, astrocyte-derived synaptogenetic compounds will be discussed. This research will provide an expanded look at the molecular pathways that glial cells use to inform synaptogenesis *in vivo*.

880S **Sexually dimorphic regulation of central synapse development by Neurexin and Neuroligins** Kristen C Davis, Timothy MoscaNeuroscience, Thomas Jefferson University

Efficient function of neural circuits requires the formation of synapses at exact times, in a precise order, and between specific partners; much of which is incompletely understood. As the development of synapses is altered in

developmental and psychiatric disorders, understanding the genes that influence synaptic development and neurological disorders like autism spectrum disorders (ASD) and schizophrenia are a major goal. Two gene families that unite synapse development with neurodevelopmental disease are the *neurexins* (*nrx*) and *neuroligins* (*nlg*). These transsynaptic cell surface molecules interact to organize synapses and mutations in these genes are linked to ASD. Nrx recruits synaptic vesicles to the presynaptic active zone and binds across the synapse to Nlg, which recruits neurotransmitter receptors to the postsynaptic membrane to receive signals. Many developmental questions remain regarding where Nrx and Nlg function, how they interact, and when they act to promote synapse formation. To understand Nrx and Nlg in the *Drosophila* CNS, we used a systematic approach to study all five *Drosophila* homologues (*dnrx* and *dnlg1-4*) in the antennal lobe (AL), a model system for studying synaptic organization and development using cell-type specific genetic labeling and manipulation. We found that *dnrx* and *dnlgs* are expressed in the AL, with differences in *dnlg3* and *dnlg4* expression between males and females, suggesting sex-specific variations in Nrx and Nlg. We used tissue-specific RNAi in presynaptic olfactory receptor neurons (ORNs) or postsynaptic projection neurons (PNs) and found a role for presynaptic Nlgs in synapse development. This role was sex-specific as *dnrx* and*dnlg1-4* in presynaptic ORNs regulate female synapses but only *dnrx, dnlg2*, and *dnlg4* regulate male synapses. This indicates sexual dimorphism in the molecular programs of synapse addition. When impaired postsynaptically, we found no role for postsynaptic Nrx, but sex-specific roles for *dnlgs*. Finally, d*nrx* / *dnlg* perturbation in adult stages only increased the number of synapses for specific *dnlgs*, highlighting a potential developmental shift for Nlg in synaptic development. Our data shows the first comprehensive assessment of Nrx and Nlg homologues in central synapse formation and reveals novel roles in presynaptic, sex-specific, and temporal synapse regulation. By understanding how these genes function, we can better grasp the changes that occur in developmental disorders like ASD.

881S **A conserved kinase cascade inhibits BMP signaling during synapse development** Kyle Boehm[1], Patrick McGraw[1], Joseph Marzano[1], Aidan Rodriguez[1], Kendall Cook[1], Cameron Rodriguez[1], Emily Januck[1], Kaitlin Tortorete[1], Joseph Peters[1], Polina Yagusevich[1], Amin Ghabrial[2], Pam Vanderzalm[11]Biology, John Carroll University, [2]Pathology and Cell Biology, Columbia University

The proper connectivity of the nervous system is critical for its function. This involves both correct target acquisition by migrating axons and subsequent growth and maturation of the synapse. The canonical Bone Morphogenic Protein (BMP) pathway helps coordinate this process in both vertebrates and invertebrates. The *Drosophila melanogaster* neuromuscular junction (NMJ) is a glutamatergic synapse used as a model for AMPA-type excitatory synapses of the mammalian central nervous system, which develop in a structurally similar way. Retrograde BMP signaling from postsynaptic muscle to presynaptic neuron is critical for the scaling growth of the synaptic termini proportional to larval muscle growth. In the absence of any BMP signaling, NMJs severely undergrow and do not release neurotransmitter normally, leading to impaired locomotion.

BMP signaling controls synaptic growth and function through activation of both canonical (transcriptional) and non-transcriptional pathways and our understanding of the regulation of canonical BMP signaling remains limited. We identified Tao, a conserved serine/threonine kinase implicated in autism, as an inhibitor of retrograde BMP signaling during the scaling growth of the larval NMJ. Tao normally inhibits NMJ growth; its neuronal loss results in overgrowth of synaptic termini without affecting muscle size. *Tao* acts in a BMP-pathway dependent manner and *Tao* loss increases the amount of pMad in motoneuron nuclei. Previous studies showed that Tao inhibits growth of epithelial tissues via activation of the conserved Hippo pathway, as a kinase functioning upstream of Hippo itself. In NMJ development, however, Tao functions independently of Hippo signaling. Here we will present the identities of the kinases downstream of Tao functioning in a Hippo-like kinase cascade and will also provide preliminary evidence for their scaffolding partners. We believe this evolutionarily conserved kinase cascade acts as a signaling cassette to inhibit the transcription of BMP target genes during NMJ development.

882V **Early-life nutrition interacts with developmental genes to shape the brain and sleep behavior in *Drosophila melanogaster*** Gonzalo H Olivares[1,2], Franco Nuñez[3], Noemi Candia[4], Nolberto Zúñiga[5], Franco Vega-Macaya[6], Karen Oróstica[7], M. Constanza González-Ramírez[8], Carlos Oliva[8], Trudy F. C Mackay[9], Ricardo A Verdugo[7,10], Patricio Olguin[111]Departamento de Neurociencia, Universidad de Chile, [2]Escuela de Kinseiología, Facultad de Medicina y Ciencias de la Salud, Universidad Mayor, [3]Escuela de Kinesiología, Universidad Mayor, [4]Departamento de Neurociencia, Programa de Genética Humana, ICBM, Universidad de Chile, [5]Facultad de Química y Biología, Universidad de Santiago, [6]Laboratorio de Genética Molecular, Universidad de Santiago de Chile, [7]Instituto de Investigación Interdisciplinaria, Vicerrectoría Académica, Universidad de Talca, [8]Department of Cellular and Molecular Biology, Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, [9]Center of Human Genetics, Clemson University, [10]Department of Basic and Clinical

Oncology, Facultad de Medicina, Universidad de Chile, [11]Universidad de Chile

The mechanisms by which the genotype interacts with nutrition during development to contribute to the variation of complex behaviors and brain morphology of adults are not well understood. Here we use the *Drosophila* Genetic Reference Panel to identify genes and pathways underlying these interactions in sleep behavior and mushroom body morphology. We show that early-life nutritional restriction has genotype-specific effects on variation in sleep behavior and brain morphology. We mapped genes associated with sleep sensitivity to early-life nutrition, which were enriched for protein-protein interactions responsible for translation, endocytosis regulation, ubiquitination, lipid metabolism, and neural development. By manipulating the expression of candidate genes in the mushroom bodies and all neurons, we confirm that genes regulating neural development, translation and insulin signaling contribute to the variable response of sleep and brain morphology to early-life nutrition. We show that the interaction between differential expression of candidate genes with nutritional restriction in early life resides in the mushroom bodies or other neurons, and that these effects are sex specific. Natural variation in genes that control the systemic response to nutrition and brain development and function interact with early-life nutrition in different types of neurons to contribute to the variation of brain morphology and adult sleep behavior.

883V **Cut homeodomain transcription factor is a novel regulator of cortical glia morphogenesis and niche maintenance around neural stem cells** VAISHALI YADAV, Richa Arya, Papri Das, Ramkrishna mishrazoology, Institute of science

Cortical glia in *Drosophila* central nervous system forms a niche around neural stem cells (NSCs) and their progeny for necessary signals to establish cross-talk with their surroundings. These cells grow and expand their thin cytoplasmic extensions around neural cell bodies in the nervous system. Although essential for the development and function of the nervous system, how these cells make the extensive and intricate membrane networks remains largely unknown. Here we show that Cut, a homeodomain transcription factor, directly regulates the fate of the cortical glia cells impacting NSC homeostasis. Focusing on thoracic ventral nerve cord (tVNC), we found that Cut is required for normal growth and development of cortical glial cells. We also highlight that levels of Cut expression play important role in the cytoplasmic network growth around the neural cells. Loss of Cut in cortical glia results in a substantial reduction in their cytoplasmic extensions and network around cell bodies of NSCs and their progeny; whereas, its overexpression induces the overall growth of cortical glia main branches at the expense of side ones. We also note a striking gain in the nuclear size and volume of cortical glial cells upon Cut overexpression. Furthermore, constitutively high Cut levels increases DNA content in these cells more than threefold indicating an interference with splitting of nuclei during endomitosis. Since cortical glia make syncytial membrane networks around neural cells, the finding identifies Cut as a regulator of glial growth and endomitosis to support a functional nervous system. This is the first report that highlights a novel function of Cut in regulating the growth and branching of cortical glial cells and control over endomitosis.

884V **Identification of cis-regulatory elements of temporal transcription factors of Drosophila medulla neuroblasts through scATAC-seq** Hailun Zhu[1], Sihai Dave Zhao[2], Alokananda Ray[1], Yu Zhang[1], Xin Li[11]Department of Cell & Developmental Biology, University of Illinois Urbana-Champaign, [2]Department of Statistics, University of Illinois Urbana-Champaign

During development, neural progenitors are temporally patterned to sequentially generate distinct neural types. Previous studies showed that sequential expression of five temporal transcription factors (TTFs), Homothorax (Hth), Eyeless (Ey), Sloppy paired (Slp), Dichaete (D) and Tailless (Tll), in medulla neuroblasts (NBs) of Drosophila larval brain is necessary for generating the full spectrum of neurons in a defined order. To characterize the complete TTF sequence and identify additional temporal patterning regulators, we applied single cell RNA sequencing (scRNA-seq) to our model system to discover all unknown TTFs and additional determinants, as well as to get a global view of the dynamic temporal patterning process of medulla neuroblasts. Our study revealed a comprehensive temporal patterning cascade: Hth + SoxN + dmrt99B -> Opa -> Ey+Erm -> Ey+Opa -> Slp+Scro -> D -> B-H1&2->Tll, Gcm, which controls the sequential generation of different neural types by regulating the expression of specific neuronal TFs. With Dmrt99B, Opa and Gcm discovered as TTFs, the mechanisms for the initiation and termination of the temporal cascade were uncovered. Moreover, we found that Lola isoforms expressed in all NBs regulate the speed of temporal progression. The transcriptional regulation requires not only the trans-regulatory elements, but also cis-regulatory elements. The result that the normal medulla TTF cascade requires Osa, a subunit of chromatin remodeling BAP complex, suggests the involvement of chromatin accessibility change in temporal patterning. By applying single-cell sequencing assay for transposase-accessible chromatin (scATAC-seq) to our model system, we identified the potential enhancers of TTFs and

assessed the dynamic chromatin accessibility of the medulla NBs. Accessible DNA sequences at different TTF gene loci are revealed to be sequentially opened as NBs age. We used those DNA sequences individually to drive the expression of a GFP reporter. The initiation of GFP expression starts at the same time as that of wild type TTFs, supporting that those DNA sequences are potentially TTF enhancers. By combining scRNA-seq and scATAC-seq data, we start to understand the regulation of TTFs at the transcriptional level.

885V **Combinatorial regulation of compartment-specific targeting of dendrites in the central brain by guidance molecules** Sijun Zhu[1], Xiaobing Deng[2], Isaac Cervantes Sandoval[31]SUNY Upstate Medical University, [2]Department of Neuroscience and Physiology, SUNY Upstate Medical University, [3]Department of Biology, Georgetown University

Establishing precise neuronal connections requires targeting of axons and dendrites not only to specific regions in the brain to find correct target cells but also to specific subcellular domains of dendrites or axons of target cells. Such subcellular specificity of neuronal connections has profound impact on neuronal activity and behavior output. However, the mechanisms regulating subcellular-specific targeting of dendrites and axons are not well understood. In the *Drosophila* mushroom body (MB), the olfactory-associative learning and memory center, individual MB output neurons (MBONs) target their dendrites to specific segments (or compartments) of MB axonal lobes to form synaptic contacts with MB axons. Meanwhile, local dopaminergic neurons (DANs) also project their axons to specific compartments of MB axonal lobes to modulate the synaptic transmission from MB neurons to MBONs and behavioral outputs by forming synaptic contacts with MB axons and MBON dendrites. Here, we investigated the cellular and molecular mechanisms underlying the compartment-specific targeting of MBON dendrites and DAN axons. Our work reveals that compartment-specific targeting of MBON dendrites and DAN axons involves mutual repulsive interactions between neighboring compartments. The repulsion can be mediated by either MBONs or DANs. The repulsive mechanisms likely act together with other mechanisms such as attractive interactions between MB axons and MBON dendrites/DAN axons and/or between MBON dendrites and DAN axons. However, mechanisms regulating compartment-specific targeting of MBON dendrites and DAN axons in the adult brains are likely different from those in the larval brains. Our work further identified two repulsive molecules Slit and Ephrin that act combinatorially to mediate repulsive interactions between neighboring compartments. In the absence of either Slit or Robo, MBON dendrites expand into neighboring compartments and form ectopic synaptic contacts with DAN axons in the neighboring compartments instead of only their cognate DAN axons within the same compartments. Our preliminary studies showed that defects in compartment-specific targeting of MBON dendrites could impact olfactory associative learning and memory. Together, our findings reveal that Slit and Ephrin-mediated repulsion is critical for restricting the growth of MBON dendrites and DAN axons to their specific compartments in the MB axonal lobes, which ensures precise connections between MBON dendrites and their cognate DAN axons in individual compartments and proper functioning of the brain.

886V **Using *Drosophila* cuticle melanization to dissect dopamine metabolism and identify novel regulators of dopamine** Samantha Deal[1,2], Danqing Bei[2,3], Shelley B Gibson[2,3], Harim Delgato-Seo[2,4], Elaine S Seto[5], Shinya Yamamoto[1,21]Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, [3]Molecular and Human Genetics, Baylor College of Medicine, [4]Department of Neuroscience, Baylor College of Medicine, [5]Texas Children's Hospital

The dopaminergic system has been extensively studied for its role in behavior as well as human neuropsychiatric and neurological diseases, but we still know little about the regulation of dopamine metabolism. In order to find new regulators of dopamine metabolism, we performed an RNAi-based screen in *Drosophila melanogaster*. Since flies use dopamine not only in the brain but also as a precursor to melanin in the cuticle, we tested for potential dopamine regulators through pigmentation defects. We identified 152 genes that altered cuticle pigmentation. These candidates were not only enriched for conserved homologs (1.3X) and disease-associated genes (1.3X), they were enriched for multiple developmental signaling pathways (e.g. *hpo*, *wts*, *Egfr*, *Sos*, *Raf*) and mitochondria-associated proteins (e.g. *COX5A*, *sesB*, *clu*, *ND-42*). This was unexpected since mitochondria have no known role in dopamine metabolism in the fruit fly. We prioritized 36 candidates for further analysis based on the strength of the pigmentation phenotype, severity of behavioral deficits, and human neurological disease association. Of these 36 candidates, 11 genes showed significantly altered levels of dopamine in the head with ten showing a reduction and one an increase, suggesting there may be a selective pressure to prevent elevated levels of total dopamine. Furthermore, of these 11 genes, three genes (*Bsg*, *clu*, and *mask*) showed a significant reduction in total dopamine in the brain. Expression analysis revealed that while many of our genes of interest are expressed in the brain, these three showed broad expression encompassing most if not all dopaminergic neurons. Of particular note, *mask*, may be a central player in dopamine regulation since it is associated with three nodes found in our screen (RTK/*Egf* signaling, *hpo* signaling, and mitochondrial dynamics). Moreover, rare

variants in the human homolog for *mask*, *ANKRD17*, have been linked to a neurological syndrome. In conclusion, this screen not only identified potential new regulators of dopamine and provided insight into the dynamics of dopamine metabolism, it also revealed an unexpected equivalence between fly pigmentation and developmental signaling or potentially human disease.

887V **The RNA binding protein Nab2 regulates splicing of RhoGEF *trio* isoforms to govern axon development** Carly L Lancaster, Pranav S Yalamanchili, Ken H Moberg, Anita H. CorbettCell Biology, Emory University

Inherited forms of intellectual disability (ID) are common in the general population and have been linked to lesions in >700 genes. Emerging evidence suggests that this diverse group of genes converge on a limited set of neurodevelopmental pathways, including those that rely on RNA binding proteins (RBPs) to guide spatiotemporal patterns of neuronal mRNA expression. Our labs co-discovered a monogenic form of ID caused by loss-of-function mutations in the ubiquitously expressed RBP ZC3H14. By probing function of the conserved ZC3H14 ortholog in *Drosophila*, Nab2, we have found that Nab2 localizes to neuronal nuclei and cytoplasmic ribonucleoprotein granules and is required specifically within brain neurons for olfactory memory and proper patterns of axon projection. At a molecular level, Nab2 can act as a translational repressor in conjunction with the Fragile-X mental retardation protein homolog Fmr1 and shares target RNAs with the Fmr1-interacting RBP Ataxin-2. However, neuronal signaling pathways regulated by Nab2, as well as mechanisms that elevate ZC3H14/Nab2 function in neurons relative to other cell types, remain elusive. We will present evidence that Nab2 controls neuronal expression of 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. 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 N^6-methyladenosine (m^6A) deposition on the *trio* pre-mRNA. Data will be presented on the role of the m^6A transferase Mettl3 and Nab2 in controlling Trio splicing and expression, and downstream effects of Nab2 loss on Trio protein and GEF activity in the mushroom body axons. Given that human TRIO is mutated in a dominant form of ID, this potential link between Nab2 and Trio in *Drosophila* could suggest that Nab2/ZC3H14 and Trio/TRIO act in a conserved ID pathway required to pattern neuronal processes in the developing nervous system.

888V **Impact of Neural Lamella Breakdown on Glial Proliferation and Eclosion Behavior** Nelchi Prashali[1], Jackson Riffe[1], Joyce Fernandes[21]Biology, Pearson Hall, Miami University, [2]Miami University

In most higher organisms, the nervous system is modified throughout the life cycle to accommodate changing behaviors or in response to injury and aging. Our research group studies how nerve pathways are remodeled during metamorphosis, a four-day period, during which the nervous system undergoes many structural alterations that form the basis for adult form and function. Five of the eight pairs of abdominal nerves converge during metamorphosis to form the terminal nerve trunk (TNT). The TNT facilitates motor behaviors in adult flies. Each abdominal nerve is ensheathed by an external acellular layer called the neural lamella (NL) and three inner layers of glial cells. Our lab is interested in the cellular and molecular mechanisms that underlie reorganization of the ensheathing layers as the nerves fuse to give rise to the TNT.

During the first day of metamorphosis, NL breaks down and an associated 4-fold increase in the number of adjacent perineurial glial (PG) cells is observed (Subramanian et. al 2017). This forms the basis for our working hypothesis that NL breakdown releases proliferative signals which are received by PG cells. A second hypothesis is that NL breakdown is a prerequisite for nerve fusions that give rise to the TNT, and would predict that any alterations in TNT formation would impact behaviors associated with the abdominal motor system, such as eclosion. To test the two hypotheses, NL breakdown was manipulated using the Gal4-UAS-$Gal80^{ts}$ system to spatially and temporally prevent the action of matrix metalloproteinases (MMPs) that are known to break down NL. A significant decrease in the number of proliferating PG cells per unit length of nerve (50 microns) was observed when NL degradation is disrupted during the earlier stages of metamorphosis *($P<0.05$)*. In studies that monitored eclosion, a significant decrease in emergence was noted in experimental animals *($P<0.01$)*.

These data support the hypothesis that NL breakdown contributes to PG cell proliferation and impacts the motor behavior of eclosion. Future experiments will analyze TNT structures, express a second transgene to prevent the action of MMPs, and express cell cycle transgenes to manipulate PG cells.

889T **The secreted protein NKT acts in a local mushroom body circuit to regulate sleep** Isabelle Draper, Mary Roberts, Matthew Gailloud, Rob JacksonNeuroscience, Tufts University School of Medicine

Sleep has been well characterized in Drosophila (Artiushin and Sehgal, 2017). We have previously shown that a small, secreted Ig-domain protein known as Noktochor (NKT) is expressed in both astrocytes and neurons of the adult Drosophila brain and required in both cell types for the regulation of night sleep (Sengupta et al. 2019). The neuronal requirement is in the fly mushroom body (MB), a neuronal locus that regulates sleep and wakefulness (Sitaraman et al., 2015). Here, we show that NKT is secreted, *in vivo*, from the MB cells that require its function. To begin to identify the cell type(s) that modulate sleep in response to secreted NKT, we used the Gal4/UAS bipartite system to drive expression of membrane-tethered versions of the Ig protein (tNKTs) in known neuronal sleep-regulating centers of the brain. Such tNKTs become localized to the cytoplasmic membrane of neurons where they can interact with putative receptors on which the Ig protein acts. The expression of tNKT broadly in mushroom body (MB) neurons reduced night sleep, similar to the effects of NKT knockdown or knockout. In contrast, expression of tNKT in sleep-regulating centers outside the MB had no effect on night sleep, indicating that glial and neuronal NKT both act within the MB to regulate sleep. In addition, tNKT expression in the cells requiring NKT (the MB alpha'/beta' neurons) also did not alter sleep, suggesting that there is a local MB circuit consisting of NKT-secreting and NKT-responding neurons that regulates sleep. To identify the receptor on which NKT acts, we have examined several transmembrane proteins that share Ig-domain homology with NKT. We suggest that a transmembrane receptor protein tyrosine phosphatase called Lar may serve as a receptor for NKT. Lar is expressed in the alpha and gamma neurons of the adult MB but not in the alpha'/beta' cells (Bali et al., 2022), which require NKT, consistent with a ligand-receptor relationship. Importantly, knockdown or overexpression of Lar in the MB increased or decreased sleep, respectively. These results indicate that Lar normally promotes wakefulness. We suggest that secreted NKT inhibits Lar (and wakefulness) to promote sleep.

References: Artiushin and Sehgal (2017) Current Opinion in Neurobiology 44, 243; Sitaraman et al. (2015) Current Biology 25, 2915; Sengupta et al. (2019) Current Biology 29, 2547–2554; Bali et al. (2022) https://doi.org/10.7554/eLife.71469.

890T **The Role of Integrin in Dementia** Dilean Murillo, Paul R Sabandal, Kyung-An HanBiological Sciences, University of Texas at El Paso

Dementia is a loss of cognitive functions and Alzheimer's disease is the most common cause of dementia (CDC). A key aspect of dementia is age-dependent neurodegeneration that cripples executive functions such as inhibitory control as well as learning and memory. Previous studies in our lab using unbiased genetic screening have identified 12 candidate dementia genes. One of those genes is *inflated* (*if),* which codes for alpha integrin. The overarching goal of this project is to identify how *if* contributes to dementia in an aging dependent manner. Alpha integrin is a cell adhesion molecule and is involved in many developmental processes. However, its role in aging and dementia is understudied. We found that the heterozygous *if* mutant flies exhibit dysfunctional inhibitory control in an aging-dependent manner while the hypomorphic mutants have compromised structural integrity of the mushroom body lobes that are important for learning and memory. These findings led us to hypothesize that alpha integrin in the mushroom body neurons plays a critical role in an aging-dependent loss of inhibitory control. We are currently testing this hypothesis.

891T **Divergent evolution of homologous neurons mediates the natural variation of courtship song types among *Drosophila* species** Dajia Ye, Justin Walsh, Ian Junker, Yun DingUniversity of Pennsylvania

Animals exhibit extraordinary behavioral diversity, indicating that nervous systems can evolve rapidly to encode new behavioral patterns. However, it is unclear how evolutionary processes sculpt neural circuits to give rise to the rich diversity of animal behaviors. To fill in this gap in knowledge, we take the evolution of *Drosophila* courtship behaviors as the model system to explore the neural mechanisms underlying species differences in behaviors. During courtship, male fruit flies perform a series of innate behaviors to attract females, including producing a courtship song by vibrating their wings. Male *D. melanogaster* performs two types of songs: sine song and pulse song. In contrast, *D. yakuba* and *D. santomea,* a pair of closely related species of *D. melanogaster*, has lost sine song in their common ancestor. Previous studies demonstrated that TN1, a group of male-specific *doublesex* (*dsx*) expressing neurons in the wing neuropile of the ventral nerve cord, is both sufficient and necessary for sine song generation in *D. melanogaster*. Using CRISPR/Cas9 genome editing, we generated *dsx*-GAL4 knock-ins that label TN1 neurons and found that *D. yakuba* has significantly fewer TN1 neurons than *D. melanogaster*. Expanding the analysis to five species in the *D. melanogaster* species group (*D. melanogaster*, *D. yakuba*, *D. santomea*, *D. teissieri* and *D. erecta*) further confirmed that the loss of sine song correlates with the loss of TN1 neurons across the phylogeny. Combing single-cell RNAseq and morphological comparison of TN1 neurons across species, we demonstrated that in *D. yakub*a, TN1 neurons have lost the specific cell subtype that innervates the downstream motor neuron to produce sine song in *D. melanogaster*. This anatomical change is expected to cause the loss of functional connectivity between TN1 neurons and the downstream circuits, leading to the loss of sine

song. Consistent with this result, specific activation of TN1 neurons optogenetically is no longer sufficient to elicit sine song in *D. yakuba*. This work has identified the neural correlates that encode species-specific behavior and demonstrated the functional significance of neuroanatomical changes in behavioral evolution among closely related species. We are currently investigating the developmental and molecular mechanisms that underlie cell-type specific loss of TN1 neurons during evolution.

892T **Role of the C Terminus of the *Drosophila* Vesicular Acetylcholine Transporter in Regulating its Trafficking and Function in the Central Nervous System** Katarzyna Rosikon[1], Hakeem O Lawal[21]Delaware State University, [2]Biological Sciences, Delaware State University

Acetylcholine synaptic neurotransmission is required for the regulation of essential life processes like cognition. Accordingly, defects in neuronal cholinergic signaling lead to an impairment in learning and memory, although the mechanism through which this deficit occurs is still not fully understood. The vesicular acetylcholine transporter (VAChT) mediates the packaging and transport of acetylcholine (ACh) for exocytotic release. And while much is known about the molecular machinery that regulates ACh, the precise manner in which VAChT, an essential component of ACh regulation, alters ACh-linked neuronal function remains a subject of active investigation. Using site-directed mutagenesis, we generated genetic constructs in which parts of the C-terminus of the transporter were deleted and used them as tools to investigate the function of that region of the transporter. We measured the effect of that change on VAChT trafficking *in vitro* by performing endocytosis assays in S2 cells. We report that deletions of specific regions in the VAChT C-terminus show aberrant transporter localization such that the transporter accumulates at the plasma membrane. Moreover, we performed a functional characterization of the effect of a C-terminal deletion and report that a complete loss of that region is lethal. Moreover, we present the results of a detailed measurement of the effect of that construct on locomotion and other ACh-linked behaviors. Taken together, our results represent one of the first *in vivo* demonstrations of a role for the VAChT C-terminus in ACh-mediated behaviors in *Drosophila*.

893T **Functional Role of Pharyngeal Gustatory Receptor Neurons in Food Choice Behavior** Seungyun Yu[1], Minkook Choi[2], Min Sung Choi[2], Jae Young Kwon[21]Biological Sciences, Department of Biological Sciences, Sungkyunkwan University, [2]Department of Biological Sciences, Sungkyunkwan University

The chemosensory system plays important roles in the survival of organisms by sensing the external and internal environments and accordingly maintaining homeostasis. Among the chemosensory organs, gustatory organs play major roles in making a decision on whether to intake nutritive foods which are necessary for living and growth, or avoid toxic materials which have harmful effects on organisms. We investigated the function of the dorsal pharyngeal sense organ (DPS), a major internal gustatory organ of *Drosophila* larvae, to elucidate complex food quality coding mechanisms. Using optogenetics and calcium imaging, we confirmed that several DPS neurons are important for sensing potential toxic chemicals and are hardwired to mostly aversive behaviors. For example, DP1 and DP2 responded to bitter compounds and non-neutral pH solutions, respectively, and each was sufficient to elicit aversive behaviors when artificially activated. On the contrary, DP4 responded to sugars and did not elicit aversive behaviors when artificially activated. Distinct from the other neurons, DP3 responded to several amino acids and showed both the characteristics of aversive and attractive neurons when artificially activated. Further neuronal inactivation experiments revealed that DP3 neurons are necessary for the sensing of several amino acids and eliciting either attractive or repulsive behaviors in response. This work was supported by the National Research Foundation of Korea [NRF-2021R1A2C1011696 and 2022M3E5E8017946].

894T **Single-cell transcriptome profiles of *Drosophila fruitless*-expressing neurons from both sexes** Colleen Palmateer[1], Catherina N Artikis[1], Savannah N Brovero[1], Benjamin N Friedman[1], Alexis Gresham[1], Michelle Arbeitman[21]Biomedical Sciences, Florida State University, [2]Florida State University

Drosophila melanogaster reproductive behaviors are orchestrated by *fruitless* neurons. We performed single-cell RNA-sequencing on pupal neurons that produce sex-specifically spliced *fru* transcripts (*fru P1* neurons). Uniform Manifold Approximation and Projection (UMAP) with clustering generates an atlas containing 113 clusters. While the male and female neurons overlap in UMAP space, more than half the clusters have sex-differences in neuron number, and nearly all clusters display sex-differential expression. Based on an examination of enriched marker genes, we annotate clusters as circadian clock neurons, mushroom body Kenyon cell neurons, neurotransmitter- and/or neuropeptide-producing, and those that express *doublesex*. Marker gene analyses also shows that genes that encode members of the immunoglobulin superfamily of cell adhesion molecules, transcription factors, neuropeptides, neuropeptide receptors, and Wnts have unique patterns of enriched expression across the clusters. *In vivo* spatial gene expression links to the clusters are

examined. A functional analysis of *fru P1* circadian neurons shows they have dimorphic roles in activity and period length. Given that most clusters are comprised of male and female neurons indicates that the sexes have *fru P1* neurons with common gene expression programs. Sex-specific expression is overlaid on this program, to build the potential for vastly different sex-specific behaviors.

895T **Cell-type-specific protein visualization reveals subcellular localization and dynamics of endogenous dopamine receptors** Shun Hiramatsu[1], Shu Kondo[2], Hidetaka Katow[3], Nobuhiro Yamagata[1], Hiromu Tanimoto[11]Graduate School of Life Sciences, Tohoku University, [2]Department of Biological Science and Technology, Tokyo University of Science, [3]Department of Cell Biology, New York University

Expression and localization of receptor subtypes determine cellular responses to extracellular ligand input, and they underlie the operation of neural circuit. Neurotransmitters have multiple corresponding receptors which can induce distinct responses. Dopamine can have opposing physiological roles, depending on which receptor subtype it signals through. In the fruit fly Drosophila melanogaster, Dop1R1 and Dop2R encode D1- and D2-like receptors, respectively and are reported to oppositely regulate cAMP signaling. Here, we profile the expression and subcellular localization of endogenous Dop1R1 and Dop2R in specific cell types of the mushroom body circuit. For cell-type specific visualization of endogenous proteins, we edited the receptor loci to tag them with the split GFP fragment. Strikingly, many cell types express both receptors, and they are localized to the presynaptic terminals, suggesting intricate presynaptic modulation. Furthermore, we found starvation-dependent, bidirectional modification of the receptor expression in the two distinct clusters of dopamine neurons. These results provide an insight into the spatiotemporal dopaminergic regulation of microcircuits by multiple receptor subtypes.

896T **Tango-Seq: overlaying transcriptomics on anatomy to understand neural circuits** Alison Ehrlich, Sofi Luminari, Angelina Xu, Simon Kidd, Jordan Russo, Justin BlauNYU

How does neuronal plasticity change circuit connections to control animal behavior? We are using the principal *Drosophila* clock neurons – the s-LNvs - to answer these questions. s-LNvs show 24 hour rhythms in the structure of their projections, which expand at dawn when s-LNvs fire, and retract at dusk when s-LNvs are hyperpolarized. Structural plasticity alters s-LNv synaptic connections and is required for normal circadian behavior. s-LNv plasticity is predictable, reproducible and cell-intrinsic, making it ideal to address the molecular mechanisms and biological functions of neuronal plasticity.

To identify the neurons downstream of s-LNvs, we used the trans-Tango anterograde labeling system, developed by the Barnea lab. The membrane-tethered trans-Tango ligand is expressed in a specific cell group using the Gal4/UAS system. All neurons produce the receptor for the trans-Tango ligand, but only downstream neurons that contact the ligand-expressing cells turn on a membrane-targeted reporter gene. We modified trans-Tango so that neurons downstream of s-LNvs express a nuclear-localized reporter gene, making it easy to count target neurons. For s-LNvs, we detect ~15 downstream clock neurons and ~30 non-clock neurons in each hemisphere.

The nuclear reporter gene also facilitates sorting of downstream target neurons. We purified s-LNv target neurons from the entire central brain, and then used single cell sequencing to analyze their transcriptomes. We call this method Tango-Seq, and it works even when starting with low cell numbers. Our results reveal that s-LNvs make sparse connections with the different subsets of clock neurons, and with other neurons that can be identified by their transcriptome. We are testing how these patterns of connectivity change during s-LNv plasticity, and identifying how s-LNvs remake predictable connections every 24 hours.

897T **Olfactory avoidance of toxic volatile electrophiles is mediated by a broadly tuned olfactory receptor in Drosophila** teruyuki matsunaga[1], Carolina E Reisenman[2], David Tadres[3], Hiromu C Suzuki[2], Santiago R Ramírez[4], Matthieu Louis[3], Noah K Whiteman[21]The University of Tokyo, [2]UC Berkeley, [3]UC Santa Barbara, [4]UC Davis

Plant-derived electrophilic toxins are detected in the diet of both fruit flies and humans by the pain receptor TrpA1, which initiates a contact-mediated aversion response. Here we report that another sensory modality, olfaction, is necessary for behavioral avoidance of these same electrophiles when volatilized. We identified one odorant receptor protein necessary for behavioral avoidance of volatilized electrophiles. Importantly, these electrophiles, which include isothiocyanates (ITCs) from mustard plants like wasabi, are highly toxic to *Drosophila melanogaster* when volatilized. Exposure to volatile allyl ITC (AITC) killed all adults within twenty minutes of exposure. We first screened electrophile-detecting-Odorant Receptors (Ors) through exhaustive electrophysiology on the whole olfactory organs in

D. melanogaster, and once candidates were identified, by heterologous expression of these Ors. We then conducted a behavioral avoidance assay and found that this Or is necessary for wild-type avoidance volatile electrophiles. Finally, we took advantage of the distantly related drosophilid fly *Scaptomyza flava*, which is a specialist herbivore of mustard plants. Because mustard plants release a variety of ITCs upon tissue damage, we hypothesized that the *S. flava* has evolved an expanded repertoire of these ITC-detecting Ors than *D. melanogaster* and other microbe-feeding relatives. Consistent with this, we found that orthologs of these Ors were triplicated in *S. flava* and are more broadly tuned to the diverse ITCs than in *D. melanogaster*. We conclude that insects perceive dangerous volatile electrophiles using the olfactory sensory modality. Moreover, a niche shifts from a microbe-feeding diet to one exclusively of living mustard leaves reshaped the function of electrophile-detecting Ors rapidly as these specialist flies have adapted to a new, toxic environment.

898T **Intense light unleashes male courtship behavior in wild-type *Drosophila*** Atsushi Ueda[1], Abigayle Berg[1], Tashmit Khan[1], Shuwen Li[1], Madeleine Ruzicka[1], Ellyn Cramer[1], Chun-Fang Wu[1], Atulya Iyengar[1,21]Biology, University of Iowa, [2]Biological Sciences, University of Alabama

Studies of the *Drosophila* courtship repertoire have elucidated several principles of the neurogenetic organization of complex behavior. Based on a multi-modal integration of olfactory, gustatory, visual, and acoustic cues, males perform stereotypic patterns of chasing, courtship song production, and copulation attempts. Here we report a serendipitous finding that intense light (~18 klx, comparable to direct sunlight) induces courtship associated motor activity in wild-type (WT) male flies, including chasing with wing extension and song production. The intensified male courtship behavior was directed to both male and female targets, as shown in both male-only and mixed sex arenas. However, in male-only chambers, we found extreme behavioral manifestations such as “chaining” and “wheeling”, which resembled previously reported male-male courtship behaviors in certain mini-*white*$^{+}$ constructs and *fruitless* mutants. Investigations into the required sensory systems, yielded results of strong implications. In visual system mutants with defective phototransduction pathway (*norpA*), the phenomenon was absent. Even in *sevenless* mutants, where the high-acuity blue/UV channels are disrupted but motion sensitivity is preserved, light-triggered courtship was greatly attenuated. However, light-induced courtship was unhampered in flies lacking acoustic signals (with wings cut to disrupt song production). Strikingly, we observed unrestrained male-male courtship behavior in olfactory mutants (*orco* and *sbl*). Particularly, *orco* males displayed maximum courtship scores regardless of light intensity. Together our observations support the notion that male courtship is restrained by olfactory cues and can be unleashed by strong visual stimulation in *Drosophila*.

899T **Sex Differences in the Reproductive Response to Energy Deficits are Reversed by Masculinizing Parts of the Female Body, But Not the Brain** Attilio Ceretti, Nacoya Madden, Jill E SchneiderBiological Sciences, Lehigh University

There are sex differences in the reproductive response to energy deficits in most species studied (reviewed by Schneider et al., 2013), but the mechanisms remain elusive. To identify the tissue responsible for these responses, we took advantage of the genetic toolkit of *Drosophila melanogaster*. Male and female flies were either fed standard media or food deprived (FD) for different lengths of time before being assessed for reproduction in a chamber without food and with a well-fed opposite-sex conspecific. 48-h FD females paired with fed males showed significantly lower copulation rate than 48-h FD males paired with fed females, and in both 48-h and 24-h FD females they took significantly longer to copulate than either fed females, fed males, or FD males ($P < 0.001$). To further examine the location of the sex difference in response to energy, we used RNA-interference (RNAi) for the *Tra2* gene to masculinize only the nervous system, or only the fat body (the functional equivalent of the mammalian liver) in 2X:2A flies with a female body. Despite their fully masculinized courtship behavior, the 24-h FD, *Tra2*-RNAi females with masculinized brains significantly decreased courtship rate, courtship time, increased latency of courtship, and made fewer copulation attempts with females compared to fed *Tra2*-RNAi females and *Tra2*-RNAi males (FD or fed) ($P < 0.01$). Thus, our genetic manipulation successfully masculinized the courtship behavior of the 2X:2A females, but this did not masculinize the response to food deprivation. By contrast, 24-h FD, Tra2-RNAi females with female brains and a masculinized fat body showed no significant deficits in the latency to copulate or percentage of flies that copulate compared to fed cohorts (i.e., the females with the masculinized fat body showed a response to energy deficit like that of control males). We hypothesize that the female-typical response to energy deficit lies outside the nervous systems, perhaps in the fat body and/or other peripheral tissue.

900T **Copulation-dependent changes in histamine immunoreactivity in the male reproductive system of *Drosophila melanogaster*** Lydia Cruce[1], Megan Maynard[2], Carley Kenney[1], Martin G. Burg[31]Cell & Molecular Biology,

Grand Valley State University, [2]Psychology, Grand Valley State University, [3]Biomedical Sciences, Grand Valley State University

Previous studies have identified histamine immunoreactivity in vacuole-like compartments (VLCs) of secondary cells in the male accessory gland that is dependent on the function of the *Hdc* gene, as no histamine immunoreactivity is detected in the secondary cells of *Hdc* mutants(1). We have previously shown that histamine deficiency alters the post-mating response (PMR) of short-term female receptivity, suggesting a role for histamine that is specific to this PMR, which is dependent on secondary cell function(2). To further investigate the functional consequence of histamine in secondary cells, we have investigated the impact of sequential copulation on histamine localization in an attempt to demonstrate histamine release from secondary cells. Four day old virgin male and female flies were placed in a mating chamber until copulation occurred, after which the female was retained, and the male was either subjected to histamine immunostaining or presented with more 2 virgin females for a second copulation. This was repeated for a 3rd consecutive copulation if the second copulation was successful. Females from the mating pairs were transferred daily to freshly yeasted food vials and the number of eggs laid and progeny produced were determined, to assess these PMRs. The reproductive organs of males after copulating 1, 2, or 3 times were examined for histamine immunoreactivity to determine whether it remained in secondary cells or was translocated elsewhere. Histamine immunoreactivity in virgin males was restricted to secondary cells as earlier described (1). After one copulation, histamine immunoreactivity levels increased in the lumen of the accessory gland, with further increases after the 2nd and 3rd sequential copulation. In addition, histamine immunoreactivity was clearly found within epithelial cells lining the ejaculatory duct (ED) after the 2nd and 3rd sequential copulation. Histamine immunoreactivity detected in the ED epithelium was variable and unequal in its distribution, dissipating within days after copulation. This result demonstrates that, like other proteins that have been localized to secondary cells(3), histamine or its metabolites are released by secondary cells into the accessory gland and transmitted into the ejaculatory duct.

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901T ***NF1* loss of function alters grooming via distinct temporal effects across grooming circuits** Genesis Omana Suarez, Seth M TomchikNeuroscience and Pharmacology, University of Iowa

Neurofibromatosis type 1 (NF1) is a genetic disorder that results from the loss of function of the *NF1* gene. Individuals with NF1 are predisposed to developing tumors and cognitive deficits such as ADHD and ASD. *NF1* loss of function in *Drosophila* leads to hyperactivity and repetitive motor behaviors, which allows us to model cognitive impairments seen in patients with NF1. However, the mechanisms by which neuronal circuits modulate spontaneous grooming behavior in Nf1-deficient flies remain to be elucidated. Here we show that Nf1-deficient flies' behavioral patterns change over time, with the flies' exhibiting a lack in grooming habituation following introduction to a novel environment. Moreover, we demonstrate temporal grooming changes across different body regions that may be due to changes in neuronal excitability and/or synaptic plasticity. These results, together with other ongoing experiments, contribute to our understanding of how NF1 impacts neuronal circuitry and habituation in *Drosophila*.

902T **Ecdysone In The Blood-Brain Barrier And Male Courtship Behavior** Marium Waqar, Brigitte DauwalderBiology and Biochemistry, University of Houston

We have previously found that the physiology of the blood-brain barrier (BBB) plays an important role in the regulation of male courtship behavior. Recently, we have shown that the nuclear hormone receptor *Hr46* (*Hormone receptor-like in 46*) is required in the BBB of adult males for normal courtship in *Drosophila melanogaster* (Lama et al. 2022). *Hr46* is well-characterized in development, where it is induced by the hormone Ecdysone and is a major mediator of the Ecdysone response. Using a reporter construct, we have found that Ecdysone is present in the BBB; however, nothing is known about its potential roles in these cells. Since, *Hr46* is a major mediator of Ecdysone signaling; we hypothesize that Ecdysone is required in the BBB in the regulation of male courtship behavior. To test our hypothesis, we are using several different strategies to examine the role of Ecdysone and Ecdysone effectors in the BBB for courtship. These include the knockdown of the Ecdysone receptor (*EcR)* in mature males and lowering the levels of Ecdysone in the BBB by overexpressing an Ecdysone degrading enzyme. We are also testing the role of alternative Ecdysone effectors like *DopEcR* and an Ecdysone transporter in the BBB.

Lama, C., Love, C. R., Le, H. N., Waqar, M., Reeve, J. L., Lama, J., & Dauwalder, B. (2022). The nuclear receptor Hr46/Hr3 is required in the blood brain barrier of mature males for courtship. *PLoS Genetics*, *18*(1), 1–15. https://doi.org/10.1371/journal.pgen.1009519 903T ***Drosophila* Odorant Binding Proteins (OBPs) in the Sensory System: A Comprehensive Understanding** Keehyun Park[1], Wayessa Rahel Asefa[1], Hyungjun Choi[1], I Joon Han[2], Chaiyoung Jeong[1], Jung Yoon Jang[1], Minsung Choi[1], Jae Young Kwon[11]Department of Biological Sciences, Sungkyunkwan University, [2]School of Medicine, Sungkyunkwan University

The chemoperception system of *Drosophila melanogaster* is complex. Odorant binding proteins (OBPs) are known to play a role in chemoperception alongside chemosensory receptors. OBPs are thought to aid in transferring hydrophobic odorants in aqueous lymph solution to olfactory receptors. Many previous studies suggest that OBPs play a variety of other roles in various physiological pathways. *Drosophila* OBPs are abundantly expressed in various tissues, according to transcriptomics experiments. However, their comprehensive expression patterns and precise functions remain unknown. Accordingly, we created a *Drosophila OBP-Gal4* driver library that can be used with various UAS reporters. We completed a catalog of *Drosophila OBP-Gal4* expression patterns with these drivers, describing expression aspects in the labellum, antennae, pharynx, maxillary palp, leg, wing, brain, ventral nerve cord (VNC), and intestine. From analyzing the patterns, we discovered that some OBPs have unique patterns that appear to be related to proprioception and glial cell function. Using reporters of Gustatory receptors and accessory cells, we were able to identify which cells in the labellum express OBPs. To investigate the roles of OBPs in gustatory sensation, we developed CRISPR-Cas9 mutant lines observed in the labellum. We performed electrophysiology and behavioral experiments on these OBP mutant flies. Overall, our research contributes to a more comprehensive understanding of the *Drosophila* OBP gene family. *These authors contributed equally to this work. This work was supported by the National Research Foundation of Korea [NRF-2021R1A2C1011696 and 2022M3E5E8017946].

904T **Fatty Acid Smell, Anesthesia, and Use on Fruit Crops.** Martine Berthelot-Grosjean[1], Gerard Maniere[2], Cindy Menage[3], Ana Depetris-Chauvin[4], Marie Juge[5], Marlyse Bourgignon[5], Solena Canale[5], Marion Canale[5], Yael Grosjean[11]Center for Taste and Feeding Behavior, National Center for Scientific Research, [2]Center for Taste and Feeding Behavior, University of Burgundy, [3]Center for Taste and Feeding Behavior, Sayens, [4]Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, [5]Technopole de l'arbois, Cearitis

Odors convey important information to select a sex partner, to find a food source, or to detect a danger. Among those some volatile molecules have been shown to cause a reversible anesthesia. However, their mode of action appears still largely mysterious. Here we describe a novel property of Olfactory Receptor 47b (OR47b), on olfactory sensory neurons (OSNs) expressing male-specific transcription factor Fru^M. We found some interesting properties of a fatty acid that can be present on food sources and oviposition sites for Drosophilid species. We show that OR47b neurons projecting to VA1v glomerulus are sensitive to this odor, and that this influences *Drosophila* behavior causing a strong aversion and even a reversible anesthesia. Strikingly, males are more sensitive than females to this acid. This effect is also adult specific, since larvae are highly attracted, and never anesthetized by this odor in contrary to carboxylic acid (CO_2) or nitric oxide (NO). Furthermore, we observed that the anesthetic sex-specific effect is not displayed in a closely related species, *Drosophila suzukii*. Finally, we used this fatty acid on cherry crops and obtained a robust protection against *D. suzukii*. These findings reveal a spectacular evolutionary and stage-dependent sensitivity to some odors through a specific sensory pathway, which can be used to protect fruits in a safe manner for the ecosystem and the biodiversity.

905F **The effect of mutated HSP27 in motor neuropathy** Georgina S Martinez, Stephanie Berumen, Ricardo Bernal, Paul Sabandal, Kyung-An HanUniversity of Texas at El Paso

The Charcot-Marie-Tooth (CMT) disease is a genetic disorder that damages the peripheral nerves and causes weakness in muscle and difficulty in movement. There are multiple types of CMT and the type 2F is specifically linked to missense mutations in the small heat-shock protein HSP27. It is however unclear whether different mutations of HSP27 lead to motor neuropathy in distinct mechanisms. We address this gap in the knowledge in *Drosophila melanogaster* by investigating the flies expressing five different missense mutations (R127W, S135F, R136W, T151I, and P182) in HSP27 linked to CMT2F. For the task, we established a novel mating assay to study motor neuropathy, in which the motor neurons innervating the Muscle of Lawrence (MOL) are targeted for human wild-type or mutated HSP27 expression. We found that the flies with a subset of HSP27 mutations expressed in MOL showed highly dampened capacity for copulation. We are currently investigating the underlying cellular mechanism. This study may provide new insight into the pathological mechanism and intervention for CMT2F.

906F **Change is good: Coupling changes in homeodomain transcription factor expression with changes in Moonwalker Descending Neuron circuit connectivity and behavior in larvae and adults** Kristen Lee[1], Chris Doe[21]Institute of Neuroscience, University of Oregon, [2]University of Oregon

Foundational studies have demonstrated that combinations of homeodomain transcription factors (HDTFs) are required for the development of neuronal identity across species. Neuronal identity properties include molecular signature, biophysiological properties, morphology, anatomical location, behavioral function, and connectivity. However, many of these neuronal properties are dynamic and known to change throughout the organism's life cycle, especially during *Drosophila* metamorphosis. It is currently unknown whether the expression profile of HDTFs within a neuron is also dynamic throughout any species lifecycle. To fill in this knowledge gap, we utilize the well-characterized *Drosophila* locomotor neuronal circuit, the Moonwalker Descending Neuron (MDN) circuit, to specifically investigate whether the HDTF expression profile changes alongside the MDN circuit connectivity. In the larvae, MDN is synaptic partners with the neurons A18b and Pair1. In the adult, MDN remains synaptic partners with Pair1, but is no longer synaptic partners with A18b and is instead partners with LBL40. We hypothesize that the HDTF mediating MDN-Pair1 connectivity will be present in both larval and adult MDN. We also hypothesize that the HDTF mediating MDN-A18b connectivity will only be present in larval MDN, and the HDTF specifying MDN-LBL40 connectivity will only be present in adult MDN. To identify candidate HDTFs mediating these connections, we preformed complementary antibody and behavior screens. Supporting our hypothesizes, we found that the HDTFs expressed in MDN are different in the larvae and adult, suggesting that the neuronal HDTF expression profile changes throughout life. Additionally, when MDN is optogenetically activated, both the larvae and adult move backward. Also supporting our hypothesizes, we have found that knocking down specific HDTFs in larval and adult MDNs alters backward locomotion, suggesting that HDTFs are important regulators of MDN connectivity. Follow-up studies utilizing RNAi knockdown, over-expression transgenes, and GFP Reconstitution Across Synaptic Partners (GRASP) are characterizing the role of each candidate HDTF in MDN-Pair1, MDN-A18b and MDN-LBL40 synapse formation in larvae and adults. These data are the first to investigate the expression and function of HDTFs at more than one time point, and suggest an important role for HDTFs in developing, maintaining, and remodeling neuronal circuits throughout life.

907F **How diet-induced changes in the gut microbiome affects the mating behavior between *Drosophila sechellia* and *D. simulans*** Sofia Pogliano[1], Joseph Coolon[21]Biology, Wesleyan University, [2]Wesleyan University

Mating behavior can contribute to speciation if enough physical or behavioral differences emerge between two populations such that they always choose to mate within their group. The diet, and more specifically the diet's effects on the gut microbiome, has previously been shown to have strong effects on the reproductive isolation between two Drosophila populations. In this study we investigate the effect that eating *Morinda citrifolia* fruit has on the mating behavior of *D. sechellia* and *D. simulans*, two closely related sister species that recently diverged. The specialist *D. sechellia* feeds on the fruit of *M. citrifolia* which is toxic to other fly species, including *D. simulans*, due to the high concentration of octanoic acid. The ingestion of *M. citrifolia* fruit has been found to cause changes in the *D. sechellia* microbiome such that *Lactobacillus plantarum* is the dominant species, and *L. plantarum* has been linked to causing changes in mating preference. Therefore, it is possible that the ingestion of M. citrifolia is causing differences in the *D. sechellia* gut microbiome such that the species maintains sympatric speciation from *D. simulans* through differences in mating behavior and mate choice. This study aims to investigate whether *M. citrifolia*-induced changes in the gut microbiome are responsible for changes in the mating behavior and mate choice of *D. sechellia* and *D. simulans*. The two species were raised for multiple generations on media containing Octanoic acid, L-DOPA (another important compound found in *M. citrifolia*), and *M. citrifolia* fruit. Their microbiome species composition were tested before and after exposure to the media, and then they underwent multiple choice mating assays to assess the sexual isolation index. Then, axenically flies were created and the gut microbe was re-introduced to the flies' gut, and the multiple mating choice assays were repeated to see if it generated the same results. It is predicted that flies raised on *M. citroflia* will prefer to mate with other flies raised on *M. citrifolia*, regardless of species type.

908F **Mechanisms of D2R signaling in the blood brain barrier that regulates courtship in *Drosophila melanogaster*** Sumit Gautam[1], Cameron R Love[2], Brigitte Dauwalder[21]University of Houston, [2]Biology and Biochemistry, University of Houston

The blood brain barrier (BBB) is a highly selective cell layer that separates the nervous system and the circulating hemolymph and protects the brain from the contents of the circulating fluids that could impede neuronal function. The BBB in *Drosophila melanogaster* is comprised of two layers of glial cells, Perineural Glia (PG) and Subperineural Glia

(SPG). We have previously shown that adult feminization of BBB cells in male *Drosophila* leads to reduced courtship[1]. In a microarray screen of isolated BBB cells, we identified several male-enriched transcripts.[2] One of them encodes the *dopamine-2 like receptor (D2R).* We have found that conditional knockdown of *D2R* in adult male *Drosophila* BBB decreases courtship levels. The *D2R* receptor is highly conserved and intriguingly, this receptor has been found to act via biased signaling (via G protein or arrestin) in mammals.[3] We have previously found that signaling through G protein and arrestin is pivotal in the regulation of male courtship behavior[1]. However, the downstream signaling pathways of *D2R* in regulating courtship behavior is not clear yet. Peterson et al. have identified residues in mouse D2R mutant which are specific for G protein and arrestin signaling [3]. We hypothesized that biased signaling through D2R, through G protein or arrestin, in adult *Drosophila* BBB mediates the effect on courtship. Our data show that D2R function in the BBB is mediated by arrestin signaling.

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909F **The neural role of Toll to nonnuclear NF Kappa B signaling in the response to alcohol** Nigel Atkinson, Thilini P WijesekeraNeuroscience, The University of Texas at Austin

In the United States, approximately 5% of individuals have an alcohol-use disorder and, excluding COVID-19 infection, a third of preventable deaths are attributed to alcohol misuse. Choices for treating alcohol addiction are very limited. Only three drugs have been approved for this treatment. They are disulfiram, naltrexone, and acamprosate. Unfortunately, the success rate of treating alcohol-addicted individuals is dismal. During the first year of treatment, two-thirds of individuals have bouts of heavy drinking, while the best three-year average shows ~25% rate of recidivism. Rational treatment of alcohol-use disorders are dependent on understanding the mechanics of alcohol addiction. In mammals, behaviors associated with alcohol addiction, including a preference for drinking alcohol, have been linked to signaling through Toll-like receptor (TLR) pathways.

In flies, the Toll signaling pathway has also been shown to modulate sensitivity to sedation with ethanol vapor. In adult flies, the NFκB encoded by the *Dif* gene is an output of the Toll signaling pathway. Toll / Dif activity modulates ethanol sensitivity—increased Toll / Dif activity reduces sensitivity, while decreased Toll / Dif increases sensitivity. Because NF Kappa B is a well-known transcription factor, it has been axiomatic that its effects are produced by an action in the nucleus. However, from flies there is strong evidence that the *Dif* NF Kappa B can directly modulate the post-synaptic machinery to alter neural signaling.

Alternative splicing produces one Dif isoform that enters the nucleus and another isoform that cannot enter the nucleus but instead co-localizes with synaptic proteins. The synaptic isoform is expressed in adult mushroom bodies and affects alcohol responses and a select number of other behaviors. Here, we describe how TLR signaling through the *Dif* NF Kappa B modulates behavior.

910F **Sleep deprivation drives brainwide changes in cholinergic pre-synapse abundance in *Drosophila melanogaster*** Jacqueline Weiss[1], Mei Blundell[2], Jeff Donlea[21]Dept. of Neurobiology, UCLA, [2]Dept of Neurobiology, UCLA

Sleep is required for robust learning and memory in many animal species, including *Drosophila melanogaster*. While insufficient sleep has been linked with altered synaptic plasticity, it is unclear whether sleep loss alters synapses uniformly across different classes of neurons or different brain regions. We recently found that pre-synaptic active zone abundance is locally increased in the Mushroom bodies, a core associative memory neuropil, and that this pre-synaptic upscaling can be attributed to excitatory, cholinergic Kenyon cells. Here, we use a genetically-encoded reporter for pre-synaptic Bruchpilot (BRP) to examine brain-wide consequences of sleep loss on synaptic distribution in neurons that

produce four different neurotransmitters (acetylcholine, dopamine, GABA, and glutamate). Flies were allowed to either sleep *ad libitum* or underwent 12h of overnight mechanical sleep deprivation before dissection and whole brain confocal imaging. Before analysis, each image was aligned to a common template for segmentation and quantification of BRP across 37 central brain neuropil regions. Sleep loss increased BRP abundance in cholinergic neurons across whole brain images, but we detected little or no net change in BRP within other cell types. Surprisingly, the increased BRP abundance in cholinergic neurons was consistent across all 37 brain regions. These findings suggest that excitatory cholinergic synapses may be most plastic in response to sleep loss and that similar plasticity rules may regulate scaling across the brain during sleep loss. To test whether chronic sleep loss might drive similar trends in synaptic scaling, we also compared the BRP abundance of each neurotransmitter system in short-sleeping *insomniac*^{2} (*inc*^{2}) mutant flies compared to genetic controls. Brains from *inc* mutants, like those from mechanically sleep-deprived flies, showed the strongest BRP increase in cholinergic neurons with weaker effects in neurons that produce other neurotransmitters. Interestingly, regional analysis showed that cholinergic neurons of *inc* mutant brains showed elevated BRP across many, but not all, of the 37 segmented neuropil regions. Our findings indicate that complementary mechanical and genetic strategies to disrupt sleep both result in elevated abundance of pre-synaptic BRP in cholinergic neurons across the fly brain.

911F **The effect of social experience on gene regulation, neural activity and behavior in *Drosophila melanogaster*** Chengcheng Du[1], Jesus Sotelo Fonseca[1], Yuta Mabuchi[2], Shania Appadoo[1], Morayo Abbey-Bada[1], Nilay Yapici[2], Corbin Jones[3], Pelin Volkan[11]Duke University, [2]Cornell University, [3]UNC-Chapel Hill

Social behaviors of animals are modulated by signals from the environment. The molecular and neural circuit-based mechanisms underlying experience-dependent behavioral regulation remains poorly understood. Gene regulation plays important roles in controlling animal behaviors. Emerging evidence from both mammals and insects indicates the intimate connection between behavioral modulation, neural transcription, and neuronal activities. The *Drosophila melanogaster* is an excellent model where links among stereotyped courtship behaviors, genes and circuits have been elucidated. Transcription factors Fruitless (Fru) and Doublesex (Dsx) control innate and learned male courtship behaviors of Drosophila, respectively. At the neural circuit level, a single cluster of P1 command neurons in the brain, which is both *fruitless* (*fru*) and *doublesex* (*dsx*) positive, drive male courtship behaviors. However, how social experience regulates master genes controlling courtship at the level of transcription in different courtship circuits remains unclear. Single-pair courtship assays showed that socially isolated male flies displayed more vigorous courtship behaviors compared to group housed males. The increase in courtship vigor in isolated males was accompanied by an increase in the response of P1 command neurons in the central brain. Brain RNA-seq and RT-qPCR showed that *dsx* expressed higher in isolated male fly brains than in group housed male flies, but not *fru*. In-vivo analysis of *dsx* promoter-driven GFP expression in the brain showed an increase in *dsx* transcription levels in isolated males compared to grouped males. Given the fact that the expression of *dsx* is more restricted to the P1 region, these results indicate close connection between the expression level of *dsx* and P1 neuronal activity. In addition, disrupting pheromone receptor function in the olfactory system altered *dsx* expression in both peripheral and central nervous system. Similar changes in *dsx* expression are also seen in *fru* mutants. These results suggest that social context alters *dsx* transcription in the courtship circuits to reprogram the expression of downstream neuromodulatory genes, and ultimately modify neuronal activity and courtship behaviors.

912F **Social spacing: elucidating the neural circuitry** J. Wesley Robinson[1], Ryley T Yost[2], Abigail Bechard[1], Waliu Alaka[1], Avneet Sahota[1], Khadijat O Mosuro[1], Jade de Belle[1], Anne F Simon[11]Biology, Western University, [2]Western University

Social behaviour can be defined as a response of individuals to conspecifics and is required for organisms to survive and reproduce. As such, social behaviour relies on the perception, integration, and response to stimuli, arising from interactions or communication from other individuals. The integration of and response to these cues from others require signalling throughout neural circuitry, which at the cellular level takes place between neurons via the synapse.

The focus of our lab is to better understand the neurogenetic underpinnings of *Drosophila melanogaster* social space determination. We and others have shown that social spacing in *D. melanogaster* can be influenced by a variety of intrinsic and extrinsic factors, such as mating status, social enrichment, genes, and environmental conditions. Many genetic factors that play a part in pre- and post-synaptic regulation and signalling are important in this social behaviour. We have shown that dopaminergic signalling is important for modulating social space, while others have also implicated acetylcholine in the mushroom bodies. Recent research has also begun to connect neuropils or specific neural circuits to their associated social behaviours. Here, we show that genetic control of these circuits, and silencing specific neurotransmitter transmission, can in turn modify fly behaviour and provide insight into the neural circuitry controlling

social space.

I will present the culmination of progress our lab has made in elucidating the basis underlying the decision-making process for group formation and distancing, as represented by the number of flies within four body lengths in the social space assay. Our findings identify specific neuropil involvement in social space and the causal neural circuits for the sex-specific determination of social spacing. We also highlight novel synaptic molecular players and neurotransmitter types that influence social space.

913F **Non-Canonical Roles of Odorant Receptors in Thermosensory Nociception** Dustin E.A. Moon, Akira Sakurai, Atit A Patel, Erin N Lottes, Charleese Williams, Jamin M Letcher, Daniel N CoxNeuroscience Institute, Georgia State University

All organisms require sensory systems to transduce external stimuli into internal information. These sensory systems vary in complexity and mechanistic arrangement across species, however, organismal survival relies upon the ability to detect potentially damaging environments and respond appropriately. Nociception plays a critical role in adaptive behavioral responses to noxious stimuli, however the underlying mechanisms required to sense noxious cues remain incompletely understood, particularly in the case of thermosensory nociception to noxious cold stimuli. In a previously defined aversive cold behavioral assay, we implicated class III multidendritic (CIII md) neurons in sensing cold nociceptive stimuli and demonstrated these neurons are both necessary and sufficient to elicit a stereotypical cold-evoked contraction (CT) behavior. Our previous studies have implicated TRP channels, metabotropic $GABA_B$ signaling coupled to calcium induced calcium release, and calcium activated chloride channel activity in mediating cold-evoked CT behavior, although mutating these pathways does not entirely abolish noxious cold behavior suggesting additional receptors may be involved in this process. To this end, we have used CIII md neuron-specific transcriptomics to identify potential non-canonical thermosensory nociceptive receptors. We performed a genetic screen of promising candidates among traditional chemoreceptor families for olfaction, odorant receptors (ORs). Intriguingly, selectively mutating CIII-enriched ORs revealed a significant role of Or65a and the obligatory co-receptor Orco in cold nociceptive behavioral response. We confirmed the impairment by testing multiple RNAi and mutants for our target genes. For further insight into the mechanistic roles of Or65a and Orco, we conducted functional assays to explore the requirements for these genes in cold-evoked electrical activity and calcium dynamics of CIII md neurons. Both *Or65a* and *Orco* knockdowns reduced cold-evoked calcium levels and decreased the firing rate with the *Orco* knockdown also shifting the firing pattern away from a bursting pattern and toward tonic spiking. This work is the first to exhibit a non-canonical role of ORs in thermal nociception. Further, this research situates ORs with the closely related multimodal gustatory receptors and ionotropic receptors, which have likewise been implicated in thermosensation, revealing functional roles that extend beyond labeled-line chemosensation.

914F **Gene Knockdown of Transporters and Receptors in Blood Brain Barrier Reduces Sleep** Ashley Avila, Ava Lewandowski, Jesse Gui, Kaeun Lee, William Yang, Shirley ZhangCell Biology, Emory School of Medicine

Sleep is a requirement for many organisms to survive, and is regulated by circadian rhythms and homeostatic drivers. There are several theories as to why we sleep, one being the restorative model which states sleep is needed for the brain to restore metabolites and dispose of waste. The brain is separated from the blood by a unique cellular layer termed the blood-brain barrier(BBB), which regulates the passage of molecules to and from the brain. This project aimed to find molecules that regulate the sleep homeostatic drive through trafficking in or out of the brain. To investigate what endogenous molecules could be involved in driving sleep, we conducted an inducible RNAi screen in *Drosophila* targeting receptors and transporters at the BBB. Our results show that the knockdown of transporters and receptors at the BBB can generally reduce sleep quantity and quality.

915F **Analgesic signaling in *Drosophila* larvae** Roger Lopez-Bellido[1], Yan Wang[1], Nathaniel J Himmel[2], Daniel N Cox[2], Michael J Galko[31]Genetics, University of Texas MD Anderson Cancer Center, [2]Center for Neuromics, Georgia State University, [3]Genetics, UT MD Anderson Cancer Center

Drosophila have emerged as a powerful system to study nociception and nociceptive sensitization, with conserved genes and pathways regulating these responses. However, *Drosophila* have not been thought to possess clear orthologs of vertebrate opiate receptors and evidence for endogenous analgesic signaling pathways is scant. Here, we found that ingestion of non-lethal amounts of morphine by fly larvae induces a potent but transient analgesia that spans multiple sensory modalities (heat, touch, chemical). Fentanyl also induces analgesia at lower doses and morphine-induced mechanical analgesia is partially reversible by naloxone. Using a candidate gene approach, we found that a G-Protein

Coupled Receptor related to vertebrate somatostatin receptors, Allatostatin-C Receptor 1 (AstC-R1), is genetically required for morphine-induced thermal analgesia. Bioinformatic analysis reveals that AstC-R1 is within the only class of insect GPCRs related to vertebrate opiate/somatostatin receptors. In the CNS, AstC-R1 is expressed in VNC neurons that are closely apposed to and synaptically connected to the axonal tracts of class IV multidendritic nociceptive sensory neurons. In the PNS, AstC-R1 is not expressed in the class IV multidendritic neurons that are thermal nociceptors suggesting that its function in thermal analgesia may be central. We will report on tissue-specific RNAi and rescue experiments if this data is complete. Biochemical experiments are ongoing to determine if morphine directly binds to AstC-R1. Our results indicate that *Drosophila* can respond to opiates with what appears to be an ancestral analgesic signaling system. They further establish a new genetically tractable model for opiate-mediated analgesic signaling.

916F **A gene expression program induced by neuronal inactivity** Zhonghua Zhu, Jennifer Lennon, Tamara Sanchez Ortiz, Justin BlauDepartment of Biology, New York University

Synaptic plasticity is essential for learning and memory, while defective plasticity is associated with neurological conditions, including Schizophrenia and ASD. Therefore, plasticity is tightly regulated at the transcriptional and post-transcriptional levels in response to changes in neuronal activity.

Activity-regulated genes (ARGs), also known as immediate-early genes (IEGs), are rapidly activated via the calcium influx which occurs during neuronal firing. Are there also genes induced by hyperpolarization? We have previously shown that the Rho1 GEF *Pura* drives the daily retraction of the principal *Drosophila* circadian pacemaker neurons, the sLNvs. Here, we present data showing that a *Pura* transcriptional reporter gene is repressed by neuronal activity and induced by hyperpolarization. Transcription of *Pura* in response to neuronal inactivity requires the transcription factor Toy, whose expression is also induced by hyperpolarization. Therefore, *toy* and *Pura* are inactivity-regulated genes (IRGs). We show that *toy* transcription is induced by low intracellular calcium and repressed by directly triggering calcium influx, indicating that intracellular calcium levels represent two cellular states and act as a binary switch to control two distinct gene expression programs: high calcium induces ARGs, and low calcium induces the IRGs. In support of this, we found that inhibiting CaMKII activity also increases *toy* transcription.

I plan to identify how low CaMKII activity activates *toy* transcription, as well finding additional Toy target genes involved in synaptic downscaling. We propose that these inactivity-regulated genes are a general property of (plastic) neurons.

917F **Investigating the role of a cluster of dopaminergic neurons in the regulation of reward-driven behaviors in *Drosophila*** Yoonwoo Park[1], Madison Endres[1], Danial Bushey[2], Ulrike Heberlein[2], Lisha Shao[31]University of Delaware, [2]Howard Hughes Medical Institute, [3]Biological Sciences, University of Delaware

The motivation to consume rewards, including food and sex, depends on the internal state and the value of the rewarding stimuli. Understanding how motivation is represented and regulated in the nervous system is essential for understanding reward-driven behaviors as well as pathophysiology of mental disorders, such as depression and addiction. Dopaminergic neurons (DANs) have been shown to signal for reward value and motivation in various species. Yet the functions of DANs in reward processing remains to be fully explored due to the heterogeneity in their location, projection, and transcriptional profiles. Many clusters of DANs have been identified in the *Drosophila* brain that mediate distinctive aspects of reward processing. Recently, we identified a cluster of DANs that may involve in the animal's motivation to engage in reward-driven behaviors. Specifically, we identified two cell types in the paired posterior medial 3 (PPM3) cluster of DANs that projects to the fan-shaped body. We show that optogenetic activation of these PPM3 neurons is rewarding to the flies. Interestingly, these neurons also involve in the regulation of feeding and mating behaviors. Further research is in need to determine the role of PPM3 DANs in the regulation of reward-driven behaviors in *Drosophila*.

918F **Identification and characterization of genetic modifiers of ethanol-induced behaviors in Drosophila** Yixin Li, Xufeng SunBiology, Colby College

Alcohol (ethanol) consumption and its effects have been an inseparable part of our society and yet the mechanisms of how it affects our nervous system are poorly understood. Despite the well-known suppressive effect it has on the motor system, ethanol has also been shown to cause a bipolar response in locomotion as the concentration increases. Using Drosophila, commonly known as fruit flies, as the model organism, we investigated the effect of genetic modifiers on ethanol-induced behaviors. We overexpressed a mutant form of CHMP2B, a protein associated with frontotemporal dementia, in ellipsoid bodies and fan-shaped bodies in the Drosophila brain, which are involved in ethanol sensitivity

and tolerance. The median sedation time was manually measured in order to observe the gradual locomotion loss. Preliminary analysis suggested that expression of mutant CHMP2B in the targeted neurons resulted in delayed onset of sedation. In particular, the median sedation time for control and experimental flies is 3.5 minutes and 7 minutes, respectively. To better analyze sedation behavior, we developed an automated video tracking software, SILT, that monitors fly activity more objectively and efficiently compared to traditional sedation assay. Preliminary data from this tracker enabled us to establish baseline characteristics of various aspects of fly locomotion, such as distance traveled, velocity, and positional preference in the arena, etc. The tracker also allows us to better characterize the effect of mutant CHMP2B on ethanol-induced behaviors, informing further research on the neurological mechanisms of alcohol consumption and addiction.

919F **Assaying Learning and Memory in neuropeptide transgenics and models of disease in Drosophila** iykemroy Ikemefuna[1], Grace Curley[1], Kiel Ormerod[21]Biology, Middle Tennessee State University, [2]Biology, MTSU

Drosophila melanogaster is one of the most effective models used to answer crucial questions in biology research. This project examines learning and memory in *Drosophila* and in turn understanding how alterations of specific genes studied in the Ormerod lab can impact both brain functionality and motor behavior changes. In the Ormerod lab we examine the function of neuropeptides, assaying the effects of motor and synaptic proteins on mechanisms of neuromuscular transduction and behavior, and also modeling neurodegenerative disorders like Huntington's Disease (HD). One of the neuropeptides we look at is Octopamine which elicits its effects on the mushroom body a significant part of the *Drosophila* brain structure important for olfactory learning and memory. We first sought to develop an assay for appetitive learning in *Drosophila* larvae. Using previously established methods, we were successfully able to train third-instar larvae to associate sugar and MCH/OCT. For the training phase, 30-35 larvae were used in each assay replicate, three replicate training sessions with agarose dishes associated with the odorant MCH, and three replicate training sessions with OCT + sucrose dishes. For the testing phase the last agarose dish was used with the two odorants on opposite sides of the dish. We trained them in two different arrangements (A and B). For arrangement A the last training dish before testing was associated with OCT; for arrangement B the last training dish was with MCH. Using this approach, we obtained a preference index value of 0.3 OCT and -0.3 MCH for arrangement A and 0.2 OCT and -0.2 MCH for arrangement B using canton S, wild-type larvae. We are using this larval assay larvae to examine the impacts of altered expression of different neuropeptides and their receptors throughout the nervous system as well as examining our models for HD. We are also currently establishing an adult version of the assay to further expand our tools in this area of research.

920F **Assessing learning and memory in *Drosophila melanogaster* using an Appetitive and Aversive suppression of phototaxis assay** Thilini Wijesekera[1], Hanna Gedamu[2], Vanessa Munoz[2], Alexander Valdez[2], Nigel Atkinson[11]Neuroscience, University of Texas at Austin, [2]University of Texas at Austin

The *Drosophila melanogaster* model organism is very widely used in the study of learning and memory. We have developed a low-cost, efficient protocol to assay associative conditioning as appetitive and aversive suppression of phototaxis. We have modified the aversive suppression of phototaxis assay of Le Bourg and Buecher (2002) to facilitate batch training of flies and testing at the bench under lighted conditions. Flies are trained to associate light with an aversive stimulus. Learning is assessed by attraction or aversion to light at a T-maze assay. Additionally, we have adapted the protocol to function as an appetitive suppression of phototaxis assay, where flies are trained to show preference to the dark. The flies are collected as pupae, and batch stored as five per batch. They are batch trained to associate light with quinine in aversive suppression of phototaxis, and dark with food in appetitive suppression of phototaxis. The training is carried out by transferring the flies into quinine associated food free vials for aversive conditioning, or darkened vials containing fly food laced with apple juice for appetitive conditioning. The flies are transferred between their housing vials and training vials three times with an exposure time of three minutes per training trial. The flies are individually transferred into the T-maze to test for learning and the response of dark or light chamber choice is recorded. The T-maze is comprised of three parts of plexi-glass placed one on top of the other. The black middle section contains the T shaped corridor that leads to the light and dark choices and the center entry point for the fly. This is flanked by a clear section each at the top and bottom. The top plate has openings over the end of the two arms of the T-maze leading to dark or light chamber choices. Additionally, the apparatus has a red film above the middle section making the path visible to the experimenter while maintaining a dark environment for the fly. This facilitates performing the experiment in a well-lit environment. We explored the potential of this protocol to assay learning using wildtype Oregon R flies and successfully obtained both appetitive and aversive learning in the flies. In future studies, we plan to utilize this method to explore memory decay curves, as well as test potential learning related genes in phototaxis suppression learning and

memory.

921F **The chromatin remodeling protein Kismet and Amyloid Precursor Protein like regulate the same phenotypes at the *Drosophila* neuromuscular junction** Nicole Linskey, Emily Hendricks, Claire Kolker, Faith LieblSouthern Illinois University Edwardsville

Alzheimer's disease is characterized by plaques of amyloid β (Aβ) peptides, which are formed from processing of amyloid precursor protein (APP). These plaques can lead to compromised synaptic function, neuronal cell death, and impaired cognition. Amyloidogenic processing of APP by β-site APP-cleaving enzyme (BACE) causes Aβ to be released from the plasma membrane thereby promoting aggregation and plaque formation. Overexpression of human APP and BACE1 in *Drosophila* larval motor neurons is used as a synaptic model for late onset Alzheimer's disease. Expression of human *APP* and *BACE1* in *Drosophila* larval motor neurons produces similar phenotypes as are observed in *kismet* loss of function mutants including impaired neurotransmission and larval locomotion, deficient endocytosis, increased synaptic levels of the BMP signaling protein pMad, and increased *neuroligin2* transcripts. Kismet is the *Drosophila* ortholog of the mammalian chromatin remodeling enzymes CHD7 and CHD8. *App like* transcripts are increased in *kismet* mutant motor neurons. *Kismet* mutants do not, however, show an increase in APP like protein or Aβ. Collectively, these data suggest a model where *kismet* is a target of the transcriptional activity of APP intracellular C-terminal domain (AICD), which is also produced by BACE1 processing of APP. Alternatively, Kismet and APP like may work synergistically to regulate the same pathways. We will examine these two possibilities by assessing RNA transcripts and shared synaptic phenotypes in *kismet* and *app-like* loss of function mutants.

922S **Automated tracking of *D.melanogaster* behavioral phenotypes** Anibal Tornes Blanco[1], Tuhin S Chakraborty[2], Scott D Pletcher[31]Biological Chemistry, University of Michigan - Ann Arbor, [2]Molecular & Integrative Physiology, University of Michigan - Ann Arbor, [3]University of Michigan - Ann Arbor

The dysregulation of neural states resulting from prolonged periods of social isolation has been identified across phylogeny. The repercussions that degrees of isolation have on individuals and society at large are influencing our healthspan and lifespan in more ways than we currently understand. Consequential psychosocial stress resulting from the lack of social interaction has been linked to increased smoking, alcohol consumption, and higher mortality. Although this risk factor is well-known for decreased longevity, the neural state associated with social isolation is not understood. Using *Drosophila Melanogaster* as a model organism, we are interested in evaluating the consequences of isolation in the fruit fly neural state using highly programmable software for behavioral assays. We used the Dtrack software which uses computer vision to compile and extract real-time measurements of various fly behavior such as spatial preference, negative geotaxis, and pairwise interactions. We found that flies reared in isolation show robust behavioral phenotypes when compared with age-matched flies that were kept in a group. Using our group›s expertise in technological development and behavioral neuroscience we are working to build systems that will allow us to untangle the neuronal and molecular mechanism by which social exposure modifies a fly's behavior.

923S **Behavioral dissection of hunger states in *Drosophila*** Kristina J Weaver[1], Sonakshi Raju[2], Rachel A Rucker[3], Tuhin S Chakraborty[1], Robert A Holt[2], Scott D Pletcher[11]Department of Molecular and Integrative Physiology and Geriatrics Center, University of Michigan, [2]College of Literature, Science, and the Arts, University of Michigan, [3]Neuroscience Graduate Program, University of Michigan

Hunger is a motivational drive that promotes feeding, and it can be generated by the physiological need to consume nutrients as well as the hedonic properties of food. Brain circuits and mechanisms that regulate feeding have been described, but which of these contribute to the generation of motive forces that drive feeding is unclear. Here, we describe our first efforts at behaviorally and neuronally distinguishing hedonic from homeostatic hunger states in *Drosophila melanogaster* and propose that this system can be used as a model to dissect the molecular mechanisms that underlie feeding motivation. We visually identify and quantify behaviors exhibited by hungry flies and find that increased feeding duration is a behavioral signature of hedonic feeding motivation. Using a genetically-encoded marker of neuronal activity, we find that the mushroom body (MB) lobes are activated by hedonic food environments, and we use optogenetic inhibition to implicate a PAM>α'/β' MB circuit in hedonic feeding motivation. The identification of discrete hunger states in flies and the development of behavioral assays to measure them offers a framework to begin dissecting the molecular and circuit mechanisms that generate motivational states in the brain.

924S **A heteromeric nicotinic acetylcholine receptor promotes sleep by relaying GABAergic signals within a locus of motor and sensory integration** Tomohiro Yumita[1], Hassan Ahamed[1], Hayden Hatch[1], Ian Cossentino[1], Charalambia

Louka[1], Nicholas Stavropoulos[21]New York University School of Medicine, [2]Waksman Institute, Rutgers University

Locomotor inactivity and reduced sensory responsiveness are defining attributes of sleep, yet the underlying mechanisms are not well understood. In particular, the molecular and circuit mechanisms by which sleep-regulatory signals from the brain restrict movement and sensation remain ill-defined. Here we identify a nicotinic acetylcholine receptor (nAChR) that promotes sleep in *Drosophila* through its expression in GABAergic neurons of the ventral nerve cord (VNC), a relay for motor and sensory pathways. Biochemical, genetic, and pharmacological manipulations suggest that a heteromeric nAChR containing the α1 and β1 subunits promotes sleep by coupling cholinergic input to GABA release in the VNC and the inhibition of motor and sensory systems. The functional parallels of the VNC and the mammalian spinal cord suggest that disruptions of conserved nAChRs and analogous inhibitory circuits in humans may impair suppression of motor and sensory activity during sleep and give rise to sleep disorders.

925S **Establishing the desert-dwelling fly *Drosophila mojavensis* as a transgenic model system to study the neurobiology of thermosensory behavior** Matthew Capek, José Miguel Simões, Jessica Hua, Alessia Para, Marco GallioNeurobiology, Northwestern University

Temperature is a fundamental feature of the environment and is a key determinant of the distribution and activity of animal species. Adaptation to different thermal conditions produces a set of molecular and biochemical changes that make it possible to survive and reproduce in a given habitat. In parallel, behavior also evolves such that, as a result, cold- or hot-adapted animals not only better tolerate thermal extremes, but even prefer them. As small poikilotherms, flies of the genus *Drosophila* have adapted to live in many different climatic environments ranging from arid deserts to cold mountains. Thus, *Drosophila* are an ideal model system to study evolutionary responses to environmental temperature.

One such species is *Drosophila mojavensis*, a cactophilic fruit fly adapted to live exclusively in the deserts of southwestern United States and Mexico. Interestingly, *D. mojavensis* prefers drier and warmer conditions in comparison to its temperate-loving cousin *D. melanogaster*. However, the molecular and circuit mechanisms that underlie *D. mojavensis*' adaptation to extreme heat remain unknown.

To address this question, we are establishing *D. mojavensis* as a genetically tractable model system for the study of temperature and humidity sensing, using mutagenic and transgenic techniques such as CRISPR and transposons. Building off our work on thermosensation in *D. melanogaster*, these new genetic tools will allow us to characterize the organization of the thermosensory system in this unique extremophile fly species. Ultimately, this work may help uncover how neural circuits evolve and diversify in response to selective pressures, and provide insights into the conserved principles of sensory processing.

926S **Distinct thermometer circuits for hot and cold temperature adjust *Drosophila* behavior to different thermal conditions** Michael H Alpert, Dominic Frank, Hamin Gil, Alessia Para, Marco GallioNeurobiology, Northwestern University

Small poikilotherms such as the fruit fly, *Drosophila*, depend on absolute
temperature measurements to identify external conditions that are above (hot) or below (cold) their preferred range and to react accordingly. Hot and cold temperatures have a different impact on fly activity and sleep, but the circuits and mechanisms that adjust behavior to specific thermal conditions are not well understood. Here, we use 2-photon guided patch-clamp electrophysiology to study the relay of thermosensory information in the fly brain, and identify distinct circuits that function as a thermometers in the hot or cold range, respectively. Hot cells target and activate Lateral Posterior Neurons (LPNs) of the clock network, while the cold circuit powerfully inhibits Dorsal Neurons 1a (DN1a) through a GABA-ergic synapse. Both hot and cold circuits impact daytime sleep and activity rhythms, allowing for specific adaptations in response to hot or cold conditions. Together, our results demonstrate that the fly nervous system separately encodes and relays absolute hot and cold temperature information, show how patterns of sleep and activity can be adapted to specific temperature conditions, and illustrate how persistent drive from sensory pathways can impact behavior on extended temporal scales.

927S **Drosophila melanogaster eavesdrops on a yeast quorum-sensing signal to locate food sources** Eva Vigato[1], Drue Domagala[2], Kaitlyn Elizabeth Ellis[2], Marcus Stensmyr[3], Sophie Jeanne Cécile Caron[21]School of Biological Sciences, University of Utah, [2]University of Utah, [3]Lund University

Drosophila melanogaster and *Saccharomyces cerevisiae* feed on decaying fruits, a food source rich in sugar. It has been hypothesized that insects such as *D. melanogaster* might use volatiles produced by fermenting yeasts to locate profitable food sources, whereas yeasts might rely on the insects to disperse to new food patches. To test whether *D. melanogaster* is attracted to volatiles produced by *S. cerevisiae*, we measured the innate attraction of *D. melanogaster* for various fruit juices and fermenting products using the olfactory trap assay. We found that red wine, in particular Cabernet Sauvignon, was the most attractive product tested. Using gas chromatography–mass spectrometry, we identified the most abundant volatiles found in the headspace of Cabernet Sauvignon and tested the attraction of *Drosophila melanogaster* towards individual molecules. We found that *D. melanogaster* showed strong attraction towards 2-phenylethanol, but none of the other odorants tested. 2-phenylethanol is a quorum-sensing molecule produced by *S. cerevisiae* cultures reaching high density. We found that *D. melanogaster* are attracted by 2-phenylethanol over a wide range of concentrations, even at very high concentrations. The attraction of *D. melanogaster* to 2-phenylethanol appears to be species-specific as no other *Drosophila* species we tested displayed similar preference towards this odor in the olfactory trap assay. We found that *D. melanogaster* detect 2-phenylethanol through their OR35a-expressing receptor neurons, which connect to the VC3 projection neurons. The VC3 projection neurons further project to the lateral horn, a region processing innate olfactory-driven behaviors such as feeding. We compared the morphology of the VC3 projection neurons across different species and found species-specific patterns that could explain the observed differences in innate attraction. Altogether, these data suggest that *D. melanogaster* has evolved the ability to locate profitable food sources by eavesdropping on the chemical signals used by *S. cerevisiae* to communicate their quorum status to their conspecifics. *S. cerevisiae* might produce 2-phenylethanol not only to communicate with their conspecifics but also to recruit *D. melanogaster* as a dispersal vector to high-density habitats.

928S **Reverse engineering *Drosophila* thermotaxis** Jose Miguel Simoes[1], Richard M Suhendra[2], Joshua I. Levy[2], William M Kath[2], Alessia M Para[1], Marco M Gallio[11]Neurobiology, Northwestern University, [2]Engineering Sciences and Applied Mathematics, Northwestern University

Simple innate behavior is often described as hard-wired and largely inflexible. Here, we study the avoidance of unfavorable hot temperature in the fruit fly *Drosophila melanogaster*, a fundamental behavior and an ideal system to probe the extent to which this simple innate behavior may be hard-wired. First, we demonstrate that hot receptor neurons of the antenna and their molecular heat sensor, Gr28B.d, are essential for flies to produce thermotactic responses in a variety of thermal landscapes.

High-resolution fly tracking combined with a 3D simulation of the thermal environment shows that, in steep thermal gradients, the direction of escape turns away from heat is determined by minute temperature differences between the antennae (0.1°–1 °C). Interestingly, the antennae are also essential for normal navigation in shallow thermal gradients (where spatial differences in temperature between the left and right antenna are not present); in contrast, internal heat receptors (AC neurons) are not required for directed thermotactic responses.

Based on our measurements, we evolved a fly/vehicle model with two symmetrical sensors and motors (a “Braitenberg vehicle”) which closely approximates basic fly heat escape behavior in steep gradients. Yet critical differences between real flies and the hard-wired vehicle revealed that fly heat avoidance involves decision-making, relies on rapid learning, and is robust to new conditions. Our current work is aimed at enriching the repertoire of vehicle behavior to more closely approximate fly responses in a variety of thermal environments, the goal is to understand the neural basis for the behavioral plasticity we discovered during heat avoidance and thermotaxis.

929S **Sexual dimorphism in gut-brain signaling** Emily Gagliano[1], Ashley Bielawski[2], Erin Szalda-Petree[2], Evan MacArthur-Waltz[2], Sarah Certel[21]Neuroscience, University of Montana, [2]University of Montana

Animals must prioritize their needs to decide how to behave in any given situation. Inter-organ communication, including gut-brain signaling, plays an integral role in brain function and resulting behavioral choice. Aggression is a critical, yet energetically expensive behavior required for survival and reproduction that is also sexually-dimorphic in strategies and outcome. An often overlooked, underlying aspect of gut-brain signaling and GI physiology, is also sex, i.e., whether a cell is intrinsically male or female. Enteroendocrine cells (EE), which function as specialized secretory cells located within the *Drosophila* intestinal epithelium, exhibit sexually dimorphic changes in gene expression including genes which encode neurotransmitter receptors. Here we investigate how intestinal cellular sex impacts gut-brain communication and aggressive behavior by 1) quantifying sexually dimorphic differences in adrenergic receptor expression in EE cells, 2) identifying differences in neuropeptide levels upon adrenergic receptor manipulation, and 3) determining the role

of adrenergic receptors in communicating starved vs. fed signals in male aggression. We have found that the latency to initiate aggression is increased in males upon starvation as well as a reduction of OAa2R in EE cells. A greater understanding of sexual dimorphism in the levels and activity of neurotransmitter receptors that mediate intestinal cell signaling will help map the internal codes of social behavior and dynamics.

930S **Serotonergic Control of Feeding Microstructure in Drosophila** Ayesha Banu[1], Swetha Gowda[2], Safa Salim[2], Farhan Mohammad[21]College of Health and Life Sciences, Hamad Bin Khalifa University, [2]Hamad Bin Khalifa University

To survive, animals maintain energy homeostasis by seeking out food. Compared to freely feeding animals, food-deprived animals may choose different strategies to balance both energy and nutrition demands, per the metabolic state of the animal. Serotonin mediates internal states, modifies existing neural circuits, and regulates animal feeding behavior, including in humans and fruit flies. However, an in-depth study on the neuromodulatory effects of serotonin on feeding microstructure has been held back for several technical reasons. Firstly, most feeding assays lack the precision of manipulating neuronal activity only when animals start feeding, which does not separate neuronal effects on feeding from foraging and locomotion. Secondly, despite the availability of optogenetic tools, feeding in adult fruit flies has primarily been studied using thermogenetic systems, which are confounded with heat. Thirdly, most feeding assays have used food intake as a measurement, which has a low temporal resolution to dissect feeding at the microstructure level. To circumvent these problems, we utilized OptoPAD assay, which provides the precision of optogenetics to control neural activity contingent on the ongoing feeding behavior. We show that manipulating the serotonin circuit optogenetically affects multiple feeding parameters state-dependently. Food-deprived flies with optogenetically activated and suppressed serotonin systems feed with shorter and longer sip durations and longer and shorter inter-sip intervals, respectively. We further show that serotonin enhances and suppresses sip durations via 5-HT1B and 5-HT7 receptors, respectively.

931S **Serotonin Distinctly Controls Behavioral States in Restrained and Freely Moving *Drosophila*** Swetha BM Gowda[1], Ayesha Banu[1], Safa Salim[1], Kadir A Peker[2], Mohammad Farhan[11]Hamad Bin Khalifa University, [2]Pegasystems Inc

When trapped in a physical restraint, animals must select an escape strategy to increase their chances of survival. After falling into an inescapable trap, they react with stereotypical behaviors that differ from those displayed in escapable situations. Such behaviors involve either a wriggling response to unlock the trap or feigning death to fend off a predator attack. The neural mechanisms that regulate animal behaviors have been well-characterized for escapable situations but not for inescapable traps. We report that restrained vinegar flies exhibit alternating flailing and immobility to free themselves from the trap. We used optogenetics and intersectional genetic approaches to show that, while broader serotonin activation promotes immobility, serotonergic cells in the ventral nerve cord (VNC) regulate immobility states majorly via 5-HT7 receptors. Restrained and freely moving locomotor states are controlled by distinct mechanisms. Taken together, our study has identified serotonergic switches of the VNC that promote environment-specific adaptive behaviors.

932S **The response to fatty acids in gustatory tarsal neurons combines combinatorial and labeled-line coding in the taste system** Yunkyung Lee[1], Pavel Masek[21]Department of Cell Biology and Neuroscience Institute for Quantitative Biomedicine, Rutgers University, [2]biology, Binghamton University

The gustatory system perceives multiple taste modalities including appetitive sweet and aversive bitter. The free fatty acids (FAs) are responsible for the taste of fat in the fruit fly *Drosophila melanogaster*, as well as in mammals. We previously showed that in the proboscis of a fly, FAs are sensed by a subset of sweet-sensing neurons expressing Gustatory Receptor 64f and that the neurons expressing Ionotropic Receptor 56d mediate feeding response to medium-chain FAs, but not to short- or long-chain FAs.

Here, we record responses to a broad range of FAs from individual tarsal neurons using Ca^{2+} imaging. We measure responses from single sweet-sensing or bitter-sensing neurons for each FA and for the selection of sweet and bitter tastants. We find that the sweet tastants activate the neuronal population expressing sweet-sensing receptors and inhibit the one expressing bitter receptors. The bitter tastants act in an opposite manner. On the contrary, FAs do not elicit any inhibition but activates both, the sweet-sensing and the bitter-sensing neurons.

Each FA activates subsets of individual taste neurons with different intensity and this subset is also concentration-dependent, indicating the possibility of a combinatorial coding. Despite similar responses of small subsets of similar FAs, flies are able to discriminate between even the most similar ones when tasted by their tarsi, but not when using the

proboscis, suggesting that the tarsi and the proboscis process FAs differently. Furthermore, PCA analysis for individual FAs does not support the grouping of FAs into traditional chemical classes. The intensity of activation of sweet-sensing and/or bitter-sensing neurons by specific FAs reflects in the flies' behavioral feeding responses further supporting the notion that the individual codes are indeed behaviorally relevant.

We propose a model where FAs are perceived as a combinatorial code of activated subset of sweet and bitter sensing neurons and where flies utilize two independent gustatory systems to sense FAs: 1. the tarsi, the initial point of contact with FAs, that has high discrimination abilities but a low sensitivity, and 2. the proboscis with high sensitivity but lower identity separation.

933S **Manipulation of neuron transmission in the mushroom bodies, protocerebral bridge, and *neuroligin 3*-expressing neurons affects social behaviour** Abigail T Bechard, Wesley Robinson, Ryley Yost, Anne F SimonBiology, Western University

Social interactions between animals depend upon where they settle in relation to each other; a behaviour called social spacing. This behaviour is defined as the distance between individuals in a stable group and has been shown to be affected by life experience, genetics, and the environment. Simple interactions like social spacing that mediate and precede more complex behaviours serve as a practical means to elucidate the basic neurogenetics involved in a variety of social behaviours.

Social behaviours are determined by perceiving attractive and repulsive cues from other individuals, followed by the neural integration of these cues. A key aspect in the determination of an organism's behaviour (including social spacing) is how these cues are integrated in the brain. For example, in *D. melanogaster* the Mushroom Bodies (MB) have been demonstrated to be involved in specific behaviours like temperature preference, social approach, and food-seeking, as well as learning, and memory.

Here, we are interested in elucidating the neural circuitry which modulates social spacing at the level of brain structures. We are currently investigating whether two brain structures, the Mushroom Bodies and the Protocerebral Bridge (PB), play a role in social spacing neural circuitry. These structures are of interest because they show enrichment of Neuroligin 3 (Nlg3), a postsynaptic cell adhesion protein that we previously found to have a strong association with social spacing behaviour.

Using the Gal4/UAS system in combination with social spacing assays, we quantified the behavioural response of flies when hyperactivating or silencing neuron transmission in the MB, PB, or using a Nlg3-Trojan driver. Thus far we have found that hyperactivating the MB causes flies of both sexes to group more closely together. We will share our hypotheses for the neurogenetic interaction between Nlg3 and the neural circuitry which modulates social space.

934S **Defining behavioral gene networks for Autism Spectrum Disorder genes using sleep and circadian rhythms** Bridget Lear, Vanessa Hernandez, Kyungsun Kim, Jenna Lee, Asim Khan, Tebelechi Ketema, Madeleine Harris, Ravi AlladaNeurobiology, Northwestern University

Disrupted sleep and circadian rhythms are among the most common issues observed in individuals affected by Autism Spectrum Disorder (ASD). Sleep and circadian disruptions may also contribute to the core neurodevelopmental, cognitive, and social deficits emblematic of ASD. Understanding the mechanistic basis of sleep disruption in ASD may thus reveal novel therapeutic avenues. We have employed *Drosophila* genetic models to determine the impact of ASD risk genes on sleep and circadian behavior. We have performed transgenic RNA interference (RNAi) screening of ~150 *Drosophila* homologs of human ASD risk genes identified through the Simons Foundation SPARK research initiative (https://www.sfari.org/resource/spark/). Through this screening we have identified more than 30 ASD candidate genes that reproducibly disrupt sleep and/or circadian behaviors when knocked down either in all *Drosophila* neurons or specifically in circadian clock neurons. We have validated more than 10 candidates by demonstrating that independent RNAi lines and/or mutant alleles induce the same behavioral phenotype(s) observed in the primary screen. The candidate genes identified thus far are implicated in a range of cellular processes such as chromatin regulation, mRNA processing, and synaptic transmission. The high proportion of ASD risk genes that impact sleep or circadian behavior suggests common mechanistic links, thus we are currently examining the neurodevelopmental and molecular roles of these candidate genes. In parallel to this approach, we have also assessed how gene networks may link ASD and sleep-circadian behavior by combining RNAi screening of ASD risk genes with specific manipulations of either the E3 ubiquitin ligase *cullin3 (cul3)* or the kinase gene *CK2alpha.* Mutations in the human homologs of *cul3* and *CK2alpha*

are associated with ASD related conditions, and both genes are implicated in the regulation of circadian and/or sleep behavior in *Drosophila.* From these screens we have preliminarily identified several additional ASD risk genes (~10) that may impact sleep or rhythmicity in an allele specific manner when knocked down in combination with *cul3* or *CK2alpha* manipulations. Such approaches may reveal novel gene networks underlying sleep and circadian behavioral deficits associated with ASD.

935S **A pair of dopamine-mediated neural circuits regulating reward taste memory and associated cognitive functions in *Drosophila*** Siyuan Yang, Siyuan Yang, Pavel MasekBiological Sciences, Binghamton University

Taste memory allows animals to modulate feeding behavior in accordance with the past experience to modulate uptake of previously unknown food. We establish a single-fly appetitive taste learning assay and characterize the properties of the memory. We find that silencing the majority of dopamine neurons (DANs) using *shibire*(ts), a temperature sensitive transgene that blocks the synaptic vesicle recycling, inhibits formation of this memory. We screen a collection of split-GAL4 lines that label small clusters of dopamine, mushroom bodies (MBs) intrinsic and MB output neurons. We identify two DAN clusters: 1. a small subpopulation of PPL1 DANs innervating the MB g2a'1 lobe region and MB output neurons (MBONs) targeting the same area. 2. Subpopulation of PAM DANs and MBONs that projects to the MB a1 lobe region. Each of these circuits are indispensable for formation of the appetitive taste memory. We propose a model where the PAM neurons receive information from the sensory pathway, modulate it, and via the connecting MBONs feed it into the PPL circuit for additional processing. These findings outline a neural circuitry system underlying the formation of appetitive taste memory. To understand the neuronal function of this circuitry, we determine the temporal requirements of the neurons by silencing them at specifically only during conditioning or test phase. The temporal and selective silencing of this circuitry is further used to study more complex learning principles and cognitive functions associated with reward conditioning.

936S ***Slumber* neurons in *Drosophila* dissipate sleep drive via the memory gene *radish*** Clark Rosensweig[1], Yong-Kyu Kim[1], Sharon Zhao[1], Stephanie Lopez[1], Shiju Sisobhan[1], William Kath[2], Ravi Allada[1]1Neurobiology, Northwestern University, [2]Engineering Sciences and Applied Mathematics, Northwestern University

Sleep is thought to be regulated by both the circadian clock, which is well understood, and a poorly understood homeostatic process. Although several populations of sleep promoting neurons have been identified in *Drosophila*, there are few reports of neurons whose activity results in decreased sleep following activation (anti-rebound). Because there are many reports of neurons whose activity increases sleep drive (as evidenced by elevated sleep following activation), we hypothesized that there must be a set of neurons whose activity is sufficient to decrease sleep drive as evidenced by anti-rebound. To test this hypothesis, we expressed TrpA1, a temperature sensitive ion channel, with a library of ~500 GAL4 fly driver lines, then exposed these flies to a 12 hr temperature pulse during the daytime to exogenously activate various sets of neurons. We identified a sleep promoting line that displayed a robust anti-rebound phenotype. The line, which we have named Slumber-Gal4, expresses in a heterogeneous population of neurons in the central brain and optic lobes, robustly promotes sleep behavior, and following activation reduces nighttime sleep by more than 200 minutes. Single cell RNA-sequencing of Slumber neurons reveals that the expression pattern is composed of 20 distinct cell types. Several clusters, including a mushroom body subset, are marked by expression of a previously reported sleep promoting neuropeptide, short neuropeptide F (sNPF). Knockdown of sNPF in Slumber neurons significantly decreases baseline sleep and the anti-rebound phenotype. We hypothesized that homeostatic potentiation of Slumber synapses might underlie the dissipation of sleep drive. To test this idea, we ran a small screen to knock down genes with a role in synaptic regulation or plasticity identified in our single cell dataset. Knockdown of *radish*, a predicted Rap-like GTPase activating protein with a major role in anesthesia-resistant memory, significantly decreased the anti-rebound phenotype. We also find that activation of Slumber neurons after training enhances long term memory further supporting a role in sleep-dependent plasticity. Thus, we have identified a novel driver marking a set of sleep promoting neurons with a robust anti-rebound phenotype. Knockdown of sNPF and *radish* both block the anti-rebound phenotype suggesting that the homeostatic process is modulated by both specific signaling modalities and small GTPase signaling cascades.

937S **Characterizing the effects of altered cholinergic synaptic transmission with age** Rohina A Nemat, Hakeem O Lawal, DaShan OsborneBiological Sciences, Delaware State University

Acetylcholine (ACh) plays a central role in the regulation of key life functions including locomotion and cognition. Once synthesized in the cytoplasm, the neurotransmitter is transported to and stored in synaptic vesicles by the Vesicular Acetylcholine Transporter (VAChT). Despite a wealth of knowledge about the regulation of ACh synaptic transmission

and its role in aging, much remains poorly understood regarding how cholinergic release is mediated late in the lifespan or its precise role in behavioral decline during aging. We are interested in determining how ACh synapses, and the behaviors they mediate, are altered during aging; and the role that changes in the expression or function of VAChT may play in that process. We seek to visualize age-related changes in the expression and localization of VAChT, and measure alterations in ACh-linked behaviors. We report that increases or decreases in VAChT function cause deficits in behaviors like locomotion that are regulated in part by ACh. We also show that alterations in VAChT impair a thigmotaxis-like behavior exhibited by flies. Moreover, we present preliminary reports on our study aimed at determining the subcellular localization of VAChT in aged flies. Future studies will focus on how changes in age and/or VAChT expression alter synaptic structure and physiology. Together, this work demonstrates the relevance of central cholinergic signaling in the regulation of key behaviors during the lifespan.

938S **Possible learning and memory modulation in Drosophila mediated by the α1T channel** Maitlyn L Pezzo[1], Andrea Valenzuela[21]Biology, Pasadena City College, [2]Chemistry, Pasadena City College

Voltage gated calcium channels regulate calcium influx and vesicular release of neurotransmitter at chemical synapses. In Drosophila, there are three genes, Dmca1D, 1A, and α1T encoding Cav1, Cav2 and Cav3-type channels, respectively. The α1T channel has been shown to be expressed across the adult fly brain, including sensory neuropils, motor-associated neuropils, and neuropils associated with cognitive ability. These channels influence behavior, for example, studies have demonstrated that α1T channels are important in the regulation of sleep-wake cycles. Specifically, a lack of α1T channels has resulted in longer sleep periods than what is usually displayed in adult Drosophila. In our research, we explored the role the α1T gene in adult Drosophila plays in learning and memory functions. We assessed these functions with the application of the T-maze. Our data suggests that α1T gene manipulation results in short-term memory loss, in addition to a disruption in an ability to learn.

939V **Neurogenetic analyses of the PDF neuropeptide maturation** Gyunghee Lee, Jae Park, Kevin ZengUniversity of Tennessee

PDF neuropeptide is an important signaling molecule that controls circadian rhythms in Drosophila. Mature PDF (mPDF) is predicted to be produced from its larger precursor (preproPDF) via cleavage and amidation during intracellular traffikcing. Here we investigated individual steps in the path producing mPDF in vivo by using a combination of mutant Pdf transgenes, targeted silencing of processing enzymes, immunohisotochemistry and circadian behavioral analysis. We veryfied the roles of Amontillado (Amon)-encoded prohormone convertase for cleavage of proPDF at the consensus KR site, Silver (Svr)-endcoded carboxypeptidase D for removing basic residues, and PHM (Peptidylglycine-α-hydroxylating monooxygenase) for amidation of PDF. Uncleavable mutant proPDFK81Q is also amidated and packaged into the secretory vesicles; however, it did not rescue Pdf01 mutant behavior, indicating that the cleavage and amidation reaction are separate events. Two amidation-defective mutants (PdfK102Q and PdfG101X) also failed to rescue Pdf01, butressing the role of amidation for PDF circadian function. Interestingly, PDF or proPDF derived from the PdfK102X mutant gene is amidated and then rescued Pdf01 mutant behavior, suggeting that the terminal basic residue is indispendable. Together, these findings highlight biological significance of PAP and each processing step for the production of functional PDF neuropeptide in vivo.

940V **Understanding the impact of caffeine exposure on sleep using conditional probability** Aishwarya Segu[1], Nisha N Kannan[21]Biological Sciences, Indian Institute of Science Education and Research, Thiruvananthapuram, [2]Biological Sciences, Indian Institute of Science, Education and Research, Thiruvananthapuram

Caffeine is one of the widely consumed psycho-stimulants in the world. Caffeine intake promotes wakefulness by activating dopaminergic neurons which leads to sleep reduction in *Drosophila*. Sleep, known to be regulated by the homeostatic system and the circadian clock regulates the depth and timing of sleep respectively. In humans, caffeine is consumed on a daily basis and these affects vary with increasing age. Because sleep quality reduces with age. Hence, it is important to understand the effect of sleep in an age dependent manner. In our study, we modulated the impact of caffeine in 4 differently, aged groups of flies namely 1, 10, 20 and 30 day old flies. The results of our study showed that short treatment to caffeine across age groups reduces sleep in flies. Interestingly, sleep fragmentation is enhanced with increasing age in flies. Furthermore, this decrease in sleep was not mediated through the homeostatic pathway. To further quantitatively elucidate the effects of caffeine on sleep, we quantified sleep parameters using probabilistic methods using mathematical modelling. From our model we observed that sleep fragmentation caused by caffeine only affects sleep but does not disturb wake episodes. In summary, the results of our studies showed that short treatment to

caffeine decreases sleep, which is not mediated by homeostatic pathway and sleep fragmentation was enhanced with increasing age.

941V **Studying Gustatory receptors using the *Drosophila* Genetic Reference Panel (DGRP)** Hyungjun Choi[1], Ha Yeon Sung[2], Min Sung Choi[1], Jae Young Kwon[11]Sungkyunkwan University, [2]University of Michigan

By tasting food, animals can determine its nutritional value. Bitterness, for example, is an indicator of toxicity and causes avoidance behavior. Bitter-sensing receptors, neurons, and behavior in response to bitter chemicals have been studied extensively in *Drosophila melanogaster*. The *Drosophila melanogaster* Genetic Reference Panel (DGRP) is a population made up of over 200 inbred lines gathered from mated females in Raleigh, North Carolina. We tested 38 DGRP lines for behavioral responses to 4 kinds of bitter chemicals to see if the DGRP could be used to study gustatory receptors. Using behavioral assays and electrophysiology experiments, we found that three of the 38 DGRP lines tested could not detect or avoid L-canavanine. A comparison of the sequences of the DGRP lines revealed that the three L-canavanine-insensitive lines have polymorphisms in the *Gr98b* coding region, with one also having a mutation in the *Gr8a* coding region. Experiments with trans-heterozygote flies revealed that *Gr98b* and *Gr8a* are defective in the L-canavanine-insensitive lines. Rescue of wild-type *Gr98b* in DGRP *Gr98b* background flies completely restored the avoidance and electrophysiology responses to L-canavanine. Furthermore, rescue experiments with modified Gr98b, which shares the same polymorphism as the Gr98b of L-canavanine insensitive DGRP lines, revealed a critical region for proper L-canavanine sensing. As a result, we confirmed that the DGRP can be used to investigate tastant sensing components. This work was supported by the National Research Foundation of Korea [NRF-2021R1A2C1011696 and 2022M3E5E8017946].

942V **Functional evolution of odorant receptors in bark beetles** Jibin Johny, Souleymane Diallo, Blanka Kalinová, Ewald Große-Wilde, Fredrick SchlyterForestry and Wood Sciences, Czech University of Life Sciences Prague

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a highly diverse subfamily of weevils, considered a natural part of forest ecosystems. However, the recurrent outbreak phases categorized them as serious pests of forests with huge economic impacts. Pheromone communication is vital for bark beetle survival as they employ species-specific aggregation pheromones to attract conspecifics to their host trees for the purposes of mating and resource exploitation. A clear phylogenetic pattern has been observed in the bark beetle aggregation pheromone composition, as some components are better conserved than others. The differences in aggregation pheromone composition are greater between closely related species than with distantly related species indicating a rapid selection of certain compounds. In our study, we hypothesize similar rapid and heritable switches in the peripheral olfactory reception at the molecular level, *i.e.* in the structure and expression of odorant receptors. To study this, we generated antennal transcriptomes of closely related *Ips* species available in the Czech Republic and identified the chemosensory gene families, including olfactory receptors. This enabled the transgenic expression of these receptors in *Drosophila melanogaster* in conjunction with electrophysiological measurements for functional characterization. Our results reveal the molecular basis of peripheral olfactory communication in bark beetles and eventually could lead to the development of better pest control strategies.

943T **Tau phosphorylation mediates neurotoxicity through actin binding *in Drosophila*** Camila A Zanella[1], Mel B Feany[21]Harvard Medical School, [2]Pathology, Harvard Medical School

Currently, it is estimated that approximately 44 million people worldwide are living with Alzheimer's disease (AD) or related dementia. Age and life expectancy increases and together increase the prevalence of dementia and AD. Tau is a hallmark protein in the pathology of AD. Under physiological conditions tau stabilizes the microtubules. In the diseased brain, however, tau becomes abnormally hyperphosphorylated, which leads to microtubules destabilization, and tau accumulation. Tau-mediated neurotoxicity is highly dependent on actin cytoskeleton. It is not fully understood how the phosphorylation of tau can interfere with tau-actin interactions and trigger neurotoxicity *in vivo*. Tau is phosphorylated by a range of kinases in many phosphorylation sites including at the KXGS motifs, a critical region to regulate tau and microtubule interaction. In our study, we used a well-established genetic model of AD in *Drosophila*. In this model, flies express human wild-type form of tau in neurons. We mutated the four serine present at the KXGS motifs to alanine, to prevent phosphorylation, and evaluated different features in the fly brain and behavior. For neurotoxicity evaluation, we analyzed the following: life span, brain vacuolization and actin rods count, caspase positive neurons count, and climbing behavior. Males and female flies were used, and all flies were aged up to 10 days. Each genotype had 6 flies and the results were analyzed using one-way ANOVA followed by post hoc Tukey's HSD multiple comparison. Our results show

that mutating the 4 serine at KXGS motifs to alanine blocked tau neurotoxicity. Preventing the phosphorylation of tau at these four sites was able to rescue all features evaluated to control levels. Further, we show that a single mutation in the KXGS motif (serine 262 mutated to alanine) can prevent actin bundling *in vitro* suggesting that actin and tau interactions are critical to promote neurotoxicity. These findings increase our understanding on how phosphorylation in these motifs mediates tau neurotoxicity by regulating actin binding. Preventing tau phosphorylation at specific motifs could became a potential therapeutic pathway in the treatment of tau-mediated neurotoxicity.

944T **Characterization of membrane trafficking pathway genes as Alzheimer disease-associated genes in Korean population through functional genomics using *Drosophila* model** Byoungyun Choi[1], So-Yoon Won[1], Nazira Jamal Albargothy[2], Seokhui Jang[1], Chaejin Lim[1], Chunyu Yuan[1], Changmin Shin[1], Sarang Kang[3], Jungsoo Gim[3], Kun Ho Lee[3], Martin Hallbeck[2], Kyoung Sang Cho[11]Biological Sciences, Konkuk University, [2]Biomedical and Clinical Sciences and Clinical Pathology, Linköping University, [3]Integrative Biological Sciences and BK21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University

Alzheimer's disease (AD) is a multifactorial disease affected by various genetic factors, and it is known that the inheritance rate reaches about 70%. Although many human genetic studies have previously revealed that more than 40 genes are associated with AD, the inheritance rate that they can explain is only about 25% of the overall inheritance of LOAD. The remaining 75% of missing heritability is probably due to the common mutation of various genes, which should be discovered to account for all the inheritance rates of AD. Here, we identified 106 AD-associated genes in the Korean population by functional genomic screening with a *Drosophila* AD model. Pathway analysis revealed that membrane trafficking is the highly enriched pathway in the discovered genes. Among them, the functional correlation between 5 selected genes (*garnet*, *shibire*, *EndoA*, *Pi3K59F*, and *CG9951*) and amyloid β (Aβ) was validated. Knockdown of these genes in the *Drosophila* eyes with expressing Aβ exacerbated the rough eye and yellow or black necrosis phenotypes. Since the knockdown of these genes alone in the eyes results in little changes in most cases, these genes are thought to be associated with Aβ toxicity. Supporting this idea, silencing each of these genes increased Aβ aggregation compared to control, indicating that these genes are important for Aβ clearance. Moreover, silencing each of these genes deteriorated the Aβ-induced neuronal cell death, and neurodegeneration in the brain of *Drosophila*. The protein levels of AP3D1, DNM3, SH3GL2, PIK3C3, and CCDC22 were remarkably lowered in the brain cortex of human AD patients compared to that of age-matched control, suggesting that impairment of their functions might be important in human AD pathology. In conclusion, our results suggest that membrane trafficking is the major cellular pathway related with AD, and that the downregulation of molecular components of membrane trafficking machinery may be involved in the pathological mechanism underlying AD.

945T **Targeted downregulation of *Hipp1* ameliorates tau-induced deficits in *Drosophila melanogaster*** SUNG YEON PARK[1], Jieun Seo[1], Seulbee Lee[1], Joohyung Kim[2], Sang Jeong Kim[1], Seungbok Lee[2], Yang-Sook Chun[11]Seoul National University, College of Medicine, [2]Seoul National University

Tauopathies, such as Alzheimer's disease (AD), are neurodegenerative diseases characterized by the deposition of neurofibrillary tangles comprising hyperphosphorylated tau protein in the human brain. Given that abnormal epigenetic alterations in heterochromatin configuration have been documented in AD patients and transgenic animal models of AD, we investigated the roles of novel heterochromatin-associated interactors in tauopathies. We examined whether tissue-specific downregulation or loss-of-function alleles of heterochromatin-associated interactors can affect tau-induced neurotoxicity using transgenic flies via UAS-Gal4 binary system. Here, we found that knockdown of HP1 and insulator partner protein (*Hipp1*) ameliorates tau-induced locomotion defects, reduced lifespan, and degeneration of brain tissues. Nonetheless, RNAi-mediated reduction of *Hipp1* failed to restore tau-induced heterochromatin loosening; it accelerated abnormal overexpression of heterochromatic genes. Instead, knockdown of *Hipp1* restored tau-driven aberrant expression of putative insulator targets and aberrant insulator-mediated epigenetic alterations. HIPP1 may have a role as an insulator binding partner regarding to be implicated in tau-induced neurodegeneration. Moreover, knockdown of *Hipp1* in flies overexpressing tau restored the aberrant expression of AD susceptibility genes, *Amph* and *Sox102F*. These results suggest that downregulation of *Hipp1* expression may be a potential therapeutic target in neurodegenerative diseases; they also provide new insights regarding the roles of insulator proteins in tauopathies.

946T ***Drosophila* models for obesity-induced fibrosis reveal evidence of cardiovascular disease** Rachel M Andrews, J Roger JacobsBiology, McMaster University

Obesity is a world-wide epidemic with 30% of the population considered obese. Obesity is a known risk factor for the

development of cardiovascular disease (CVD), which is accompanied by aberrant remodeling of the cardiac extracellular matrix (ECM). The ECM is a protein and proteoglycan scaffold that is necessary for supporting heart physiology, homeostasis, and structural integrity under mechanical stress. In CVD, there is excessive deposition of extracellular matrix proteins, termed fibrosis. Fibrotic remodeling negatively affects heart function, is irreversible and progressive, and currently has no available treatments. However, this phenomenon is difficult to study *in vivo* due to the complex gene families and lethality associated with altered cardiac function present in vertebrates. This necessitates the use of a genetic model like *Drosophila melanogaster* where impaired function is not lethal. I have characterized both dietary and genetic approaches to induce obesity and overgrowth in *Drosophila* during larval growth and development, and quantified the resulting changes in cardiac ECM structure, morphology, physiology, and gene expression. Both overgrowth and obesity models result in altered triglyceride levels and lipid droplet morphology, as well as impairment of heart function, revealed in both rhythmicity and stroke volume. Parallel changes in both matrix organization and fiber characteristics were also observed, including changes in Collagen fibre distribution and morphology. Obesity models in *Drosophila* will simplify the testing of possible therapeutic tools for the treatment of fibrosis.

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947T **NMNAT promotes glioma growth through regulating NAD+ metabolism** Jiaqi Liu, Xianzun Tao, Yi Zhu, Kai Ruan, Zoraida Perez, Priyamvada Rai, Grace ZhaiUniversity of Miami Miller School of Medicine

Gliomas are highly malignant brain tumors with poor prognosis and short survival. As a critical metabolic cofactor, Nicotinamide adenine dinucleotide (NAD^+) has been shown to impact multiple processes that are dysregulated in cancer. However, anti-cancer therapies targeting NAD^+ synthesis have had limited success due to insufficient mechanistic understanding of NAD^+ metabolic pathways in glioma. Here, we adapted a *Drosophila* glial neoplasia model and discovered the genetic requirement for NAD^+ synthase nicotinamide mononucleotide adenylyltransferase (NMNAT) in glioma progression in vivo in *Drosophila* and in human glioma cells. Overexpressing enzymatically active NMNAT significantly promotes glial neoplasia growth and reduces animal viability. Mechanistic analysis suggests that NMNAT interferes with DNA damage-p53-caspase-3 apoptosis signaling pathway by enhancing NAD^+-dependent posttranslational modifications (PTMs) poly(ADP-ribosyl)ation (PARylation) and deacetylation of p53. Since PARylation and deacetylation reduce p53 pro-apoptotic activity, modulating p53 PTMs could be a key mechanism by which NMNAT promotes glioma growth. Our findings reveal a novel tumorigenic mechanism involving protein complex formation of p53 with NAD^+ synthetic enzyme NMNAT and NAD^+-dependent PTM enzymes that regulates glioma growth, and further highlight the exciting potential of *Drosophila* models in facilitating anti-cancer therapeutic design.

948T **Probing the mechanism of ROS-induced glial lipid droplet formation and implications for Alzheimer's disease** Matthew Moulton[1,2], Scott Barish[1], Isha Ralhan[3], Jinlan Chang[3], Lindsey Goodman[1,2], Jake Harland[1,2], Paul Marcogliese[4], Maria Ioannou[3,5], Hugo Bellen[1,61]Molecular & Human Genetics, Baylor College of Medicine, [2]Molecular & Human Genetics, Jan & Dan Duncan Neurological Research Institute, [3]Physiology, University of Alberta, [4]Biochemistry & Medical Genetics, University of Manitoba, [5]Cell Biology, University of Alberta, [6]Neuroscience, Baylor College of Medicine

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 Alzheimer's disease (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*), *CG34120* (*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.

949T **A Drosophila model for Mucopolysaccharidosis type IIIB (MPS IIIB)** Bibhu Simkhada, Nestor O Nazario-Yepiz, Patrick S Freymuth, Trudy F C Mackay, Robert R H AnholtCenter for Human Genetics and Department of Genetics and Biochemistry, Clemson University

Mucopolysaccharidosis type IIIB (MPS IIIB) is a rare human lysosomal storage disorder caused by defects in the lysosomal enzyme alpha-*N*-acetylglucosaminidase (NAGLU). Affected individuals appear normal at birth, with clinical symptoms appearing only at around 5 years of age. Early symptoms include hyperactivity and lack of sleep, which manifests into neural degeneration by mid-teen years, and death before the age of 30. Mutations in *NAGLU* cause accumulation of partially degraded heparan sulfate in lysosomes, but how this relates to neural degeneration is unknown. We use the functional *Drosophila melanogaster* ortholog of *NAGLU*, *CG13397*, to characterize disease models induced by gene deletion, missense (Drosophila Y160C corresponding to human Y140C), and nonsense (Drosophila W422X corresponding to human W404X) mutations. Fluorescence microscopy on mutant fly brains using Lysotracker dye reveal a significant increase in lysosomal size, characteristic of lysosomal storage diseases. Using the Drosophila Activity Monitor (DAM) system to analyze activity and sleep patterns, a climbing assay and a larval crawling assay, we find hyperactivity and lack of sleep in mutant flies. RNA sequencing of fly brains can reveal differentially expressed genes to provide mechanistic insights into MPS IIIB pathogenesis.

950T **IRE1 inhibitor STF-083010 decreases Aβ levels at the *Drosophila* neuromuscular junction.** Fatemeh Barmaleki Lighvan, Emily Hendricks, Claire Kolker, Faith LieblBiology, Southern Illinois University-Edwardsville

Amyloid-β (Aβ) aggregates are one of the hallmarks of Alzheimer's disease (AD) pathogenesis. Amyloidogenic processing of amyloid precursor protein (APP) by sequential α and β secretase activity produces Aβ fragments. Accumulation of Aβ oligomers at the synapse interferes with normal synaptic function. An abundance of misfolded proteins can lead to programmed cell death and neurodegeneration in AD due to activation of the Unfolded Protein Response (UPR). The UPR can activate three highly conserved signaling pathways including the protein kinase R-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor (ATF6) pathways. To investigate Aβ-induced UPR activation, we pharmacologically inhibited UPR in *Drosophila* AD models. *Drosophila* larvae expressing human APP and BACE (β secretase) in neurons (APP; BACE) were raised on Tauroursodeoxycholic acid (TUDCA, a chemical chaperone), the PERK inhibitor GSK2656157, and IRE1 inhibitors STF-083010 and MKC-3946. APP; BACE expressing larvae showed decreased levels of Aβ when raised on 10 µM STF-083010 and 50 µM STF-083010 for their lifetime compared to DMSO controls. Aβ levels were increased, however, after only 24-hour exposure to 10 µM STF-083010. Lifetime treatment with 10 µM STF-083010 restored motor behavior in APP; BACE expressing larvae compared to controls. We are currently examining the effects of UPR activation and additional IRE1 inhibitors on Aβ levels to determine whether IRE1 inhibition is responsible for the suppression of Aβ at the synapse. In future experiments, we will investigate lifetime treatment with 10 µM STF-083010 to determine additional synaptic phenotypes and endocytosis defects in animals expressing APP and BACE in neurons.

951T **Lipophorin Receptors Genetically Modulate Presenilin-dependent Neuronal Survival in the Aging *Drosophila* Brain** Jongkyun Kang[1], Chen Zhang[1], Yuhao Wang[2], Jian Peng[3], Bonnie Berger[2], Norbert Perrimon[4], Jie Shen[11]Neurology, Brigham and Women's Hospital, Havard Medical School, [2]Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, [3]Computer Science, University of Illinois at Urbana-Champaign, [4]Genetics, Harvard Medical School

Mutations in the *Presenilin* genes are the most common cause of familial Alzheimer's disease. Presenilin (PS) is the catalytic subunit of γ-secretase, which cleaves type I transmembrane proteins. Previous genetic studies in mice and *Drosophila* demonstrated an evolutionarily conserved role of PS in the support of neuronal survival during aging, but the molecular pathway remains unknown. In this study, we use fruit flies to look for genetic modifiers that modulate PS-dependent neuronal survival using a newly developed neuron-specific knockdown (KD) model of *Drosophila Presenilin* (*Psn*), which exhibits age-dependent neurodegeneration and increases of apoptosis. Employing machine learning-based

bioinformatics analysis, we rank ordered 641 *Drosophila* type I transmembrane proteins. Testing each of the top 25 ranked candidates using two independent RNAi lines reveals that KD of several genes involved in lipid transport and metabolism enhanced neurodegeneration in *Psn* KD flies. Specifically, neuron-specific KD of lipophorin receptors (*LpR2* or *LpR1*) and heterozygotic germline deletions of *LpR1* and *LpR2* elevated neurodegeneration in *Psn* KD flies. Furthermore, *LpR1* or *LpR2* KD and haploinsufficiency of *LpR1* and *LpR2* also induced neurodegeneration. These findings show that lipophorin receptors modulate *Psn*-dependent neuronal survival, and that altered lipid transport and metabolism contributes to neurodegeneration in the aging brain.

952T **Effects of Compound M on Parkinsonian-Like Behavior in LRRK and Park Mutant Flies** Anna Paca[1], Danielle Dircks[1], Darshika Desai[1], Christopher L Kliethermes[2], Pramod Mahajan[11]Drake University, [2]Department of Psychology and Neuroscience, Drake University

Parkinson's disease is a progressive neurodegenerative disease caused by loss of dopaminergic transmission. FDA-approved treatments for this disease reduce symptom severity by increasing the bioavailability of the dopamine. Our laboratory has been exploring in fly models of Parkinson's disease the efficacy of Compound M, a bioactive compound isolated from a plant used in Ayurvedic medicine for the treatment of Parkinson's disease. We fed varying concentrations of Compound M to wild-type flies and to two fly lines that carry mutations in genes associated with familial Parkinson's disease, LRRK and Park. These flies were then observed in assays of negative geotaxis, horizontal locomotion, feeding, and longevity. We found genotype, sex, and concentration dependent effects of Compound M in most assays, with the clearest beneficial effect found in the climbing assay. However, the effects of the LRRK and Park mutations varied across assays, which complicates the interpretation its effects. Overall, our results suggest that Compound M might be effective at treating some aspects of Parkinson's disease, and highlight the importance of using multiple behavioral and genetic models in pharmaceutical development.

953T **Quantification of the rough eye phenotype of *Drosophila* using ilastik and Flynotyper** Qasim Mujteba[1], JiHye Kim[2], Madie Chalmers[1], Minwoo Baek[2], Nam Chul Kim[21]University of Minnesota Duluth, [2]University of Minnesota

The *Drosophila melanogaster* eye has been extensively used as a model to investigate human neurodegeneration. The *Drosophila* ommatidia is a well-structured and comprehensive array of around 800 units, exhibiting a symmetrical and hexagonal pattern. This regularity and ease of observation make the *Drosophila* eye system a powerful tool to model various human neurodegenerative diseases in *Drosophila*. However, ways of quantifying such abnormal phenotypes derived by mutant proteins such as manual ranking of eye severity scores have limitations, especially when ranking weak alterations in eye morphology. To overcome these limitations, computational approaches have been developed such as Flynotyper. However, when we use a ring light to get better qualitative images accessing the intactness of individual ommatidia, the images cannot be analyzed by Flynotyper directly. Here, we describe an unbiased way to quantify rough eye phenotypes observed in *Drosophila* disease models by combining two software, ilastik and Flynotyper. By pre-processing the images with ilastik, we successfully quantify the rough eye phenotypes with images taken with a ring light.

954T ***In vivo* drug screen aims to reveal novel therapeutic targets for photo-sensitive epilepsy** Yi Hsiao[1], Fei-Yang Tzou[2], Jui-Yu Yeh[1], Wan-Syuan Lin[2], Cheng-Li Hong[2], Chih-Chiang Chan[11]National Taiwan University, [2]Physiology, National Taiwan University

Epilepsy is a chronic noncommunicable disease of the brain affecting people of all ages. Around 50 million people worldwide are living with epilepsy, making it one of the most common neurological diseases globally. Among them, 2% to 10% of epileptics have photosensitive epilepsy (PSE), in which seizures are induced upon visual stimulation. Although it is estimated that up to 70% of people with epilepsy could live seizure-free with antiepileptic drugs (AEDs), the rest of epileptics are refractory to AEDs and the treatment might even gradually worsen the symptoms. Therefore, new drug identification is in urgent need. A previous study has demonstrated that in *Drosophila* ceramide phosphoethanolamine synthase (cpes)-null mutants, cortical glial cells failed to encapsulate the neuronal cell bodies, resulting in PSE. Therefore, we generated *cpes*-knock out fly by CRISPR to recapitulate PSE. The *cpes*-KO flies display sensitivity to fluctuations in light intensity. Also, we show that the survival rate of the homozygous *cpes*-KO flies was extremely low (<0.5%), representing a modifiable phenotype. For the primary drug screen, we took advantage of the pupal lethality as a simple readout and developed a small molecule screen of about 3000 FDA-approved compounds from NIH. The candidate compounds capable of rescuing lethality in a *cpes*-KO fly model will then be examined for optimal concentration in the secondary drug screen, and we will perform behavioral assays to determine if they could suppress seizures. When the proposed experiments are completed, we will be able to identify novel drugs with therapeutic potential for PSE patients.

955T **The role of Scully in aging-related deficits in inhibitory control and memory** Paul Rafael Sabandal, Maya Solis, Carolyne Chepkosgei, Kyung-An HanThe University of Texas at El Paso

Progressive cognitive decline including impairments in inhibitory control and memory are clinical presentations of Alzheimer's disease and related dementias (ADRD). There is substantial progress in our understanding of key ADRD pathophysiological changes such as acetylcholine neuronal degeneration, mitochondria anomaly, β-amyloid aggregation and hyperphosphorylated tau accumulation but the underlying mechanisms are yet elusive. We hypothesize that the interaction of genetic and non-genetic risk factors (e.g., aging, sleep perturbation, and social stress) is important for ADRD. To test the hypothesis, we conducted an unbiased functional genetic screen to uncover novel ADRD genes that interact with non-genetic factors, specifically aging and social stress, by measuring dysfunctional inhibitory control as an endophenotype in *Drosophila*. We identified Scully, the fly homolog of 17-β-hydroxysteroid dehydrogenase 10, which is a multifunctional mitochondrial enzyme and is known to bind to Aβ peptides. The Scully-deficient flies show aging-dependent dysfunctional inhibitory control as well as augmented memory loss compared to control flies. In addition, we identified the mushroom body γ neurons as the major neural site for Scully's contribution to the aging-associated cognitive decline. We are currently working on the Scully's roles in cholinergic degeneration, mitochondrial homeostasis, amyloidogenesis, and tauopathy. Our findings will advance the knowledge of ADRD pathogenesis mechanisms and may reveal unique therapeutic targets for ADRD.

956T **Investigating the effects of *kdm5* mutations on seizure susceptibility and movement** Bethany K Terry[1], Matanel Yheskel[1], Julie Secombe[1,21]Department of Genetics, Albert Einstein College of Medicine, [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine

In humans, mutations in the Lysine (K)-specific demethylase 5 (*KDM5C)* gene have been shown to cause the X-linked intellectual disability (ID) disorder known as Claes-Jensen syndrome. Of the numerous ways in which this disorder can manifest, some reported clinical features include intellectual disability, developmental delay, gross or fine motor delays, and seizures. To better understand the function of KDM5C and the KDM5 family of histone demethylases, *Drosophila* have been used to study the role that these proteins play in neurodevelopment. Previous work has shown that loss of *Drosophila's* KDM5C ortholog *kdm5* causes changes in mushroom body development, resulting in changes in neuron morphology, neuron guidance, and cognition. How variations in the *KDM5C* or *kdm5* genes cause these changes, however, is not well understood.

In this study, we characterize the effects of patient-associated variants generated in *Drosophila kdm5* on seizure prevalence and locomotor behavior, two common areas of impact in Claes-Jensen syndrome. To do this, we examined bang-induced seizure susceptibility, heat-induced seizure susceptibility, and the movement patterns of wild-type and ID-associated allele adult flies. Ongoing analyses will utilize video recordings and movement tracking software to characterize these groups. Identification of mutations that induce seizures and locomotor changes will allow us to further investigate the mechanisms underlying these changes in the future.

957T **Traumatic brain injury induces a transposable element landscape similar to aging** Zhecheng Jin, Nancy M BoniniDepartment of Biology, University of Pennsylvania

Traumatic brain injury (TBI) is one of the major environmental risks for accelerated progression of neurodegeneration and mortality. Recent work from our lab has revealed that TBI shares a transcriptomic signature with brain aging with a focus on protein-coding genes. As a traditionally under-examined fraction of next-generation sequencing data, transposable elements (TEs) have been linked to aging and neurodegenerative disorders. We hypothesize that TEs may play a role in brain aging in response to TBI. To examine this hypothesis, we utilized an available TE-central software to quantify TEs in published TBI and aging RNA-seq datasets from *Drosophila*. We show that differentially expressed TEs in late TBI are mostly upregulated, whereas altered TE transcripts in acute TBI exhibit bidirectional changes. Among TEs de-repressed by TBI, all of them are Class I TEs. The majority of these TEs are LTR (long terminal repeat) retrotransposons, whereas the remaining are LINEs (Long interspersed nuclear elements). Comparison of TE abundance between TBI and aging shows a similar TE landscape, such that TEs dysregulated by TBI overlap those dysregulated with age. Our results suggest that perturbed transposable elements are another feature of premature brain aging that occurs upon TBI.

958T **Aldose reductase inhibitor AT-007 prevents neurodegeneration and mitochondrial dysfunction in sorbitol dehydrogenase deficiency-induced neuropathy** Amanda G Lobato[1], Yi Zhu[2], Adriana P. Rebelo[3], Tijana Canic[4], Sheyum Syed[4], Christopher Yanick[5], Mario Saporta[6], Michael Shy[7], Natalie Ortiz-Vega[8], Xianzun Tao[2], Riccardo Perfetti[9], Shoshana Shendelman[9], Stephan Zuchner[3], R. Grace Zhai[21]Graduate Program of Human Genetics and Genomics, University of

Miami, [2]Department of Molecular and Cellular Pharmacology, University of Miami, [3]Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami, [4]Department of Physics, University of Miami, [5]Graduate Program of Neuroscience, University of Miami, [6]Department of Neurology, University of Miami, [7]Department of Neurology, University of Iowa, [8]Graduate Program of Molecular and Cellular Pharmacology, University of Miami, [9]Research&Development, Applied Therapeutics

Sorbitol dehydrogenase (SORD) deficiency has been identified as the most frequent autosomal recessive form of hereditary neuropathy. Loss of SORD causes high sorbitol levels in cells due to the inability to convert sorbitol to fructose in the two-step polyol pathway, leading to neurodegeneration. However, underlying mechanisms of sorbitol-induced neurodegeneration have not been fully elucidated, and no current FDA-approved therapeutic options exist to reduce sorbitol levels in the nervous system. Here, in a *Drosophila* model of SORD deficiency, we showed synaptic degeneration in the brain and locomotor impairment. In addition, we found an accumulation of reactive oxygen species (ROS) in the central nervous system (CNS) and muscle, indicating mitochondrial dysfunction. Applied Therapeutics, Inc has developed a CNS-penetrant next-generation aldose reductase inhibitor (ARI), AT-007, which inhibits the conversion of glucose to sorbitol. AT-007 significantly reduced sorbitol levels in patient-derived fibroblasts, iPSC-derived motor neurons, and *Drosophila* brains. Moreover, feeding with AT-007 in *Drosophila* significantly mitigated synaptic degeneration, improved locomotor activity, decreased ROS levels, and improved mitochondrial morphology and ATP production. These findings establish the underlying disease pathogenesis causing inherited neuropathy due to mitochondrial impairment and provide a potential treatment strategy for patients with SORD deficiency.

959T **Development of a Model for Peanut Allergy in *Drosophila melanogaster*** Alexis M Hobbs[1], Adelaide Buhlke[2], Carlos Hernandez[3], Joseph Dolence[1], Kimberly A Carlson[11]Biology, University of Nebraska at Kearney, [2]Central City Senior High School, [3]University of Nebraska at Kearney

Peanut (PN) allergy is a common and often life-threatening food allergy in the United States. Mice are traditionally used to study PN allergy, but this can be expensive and time-consuming. *Drosophila melanogaster* have proven useful to study human diseases and disorders due to their similar genome. The objective of this study was to determine if immune regulated genes within the *D. melanogaster* genome were affected by exposure to PN. One hundred virgin females were placed into 8 pint cages. The females were reared on standard cornmeal-molasses food with either water or a 5% PN solution added to the top. Every 72 hours, the food was replaced, and the dead collected. Longevity analysis via Kaplan Meyer detected no significant differences between flies exposed to peanut and controls. qRT-PCR was performed on 3 different days across the lifespan of the flies. Preliminary results demonstrate a significant down-regulation of both *Dorsal (Dl)* and *Dif* and an up-regulation of *Cactus (Cact)* and *Relish (Rel).* Taken together, these data suggest that the Toll pathway is possibly involved in allergic reaction. This is the first demonstration that *D. melanogaster* elicits an immune response to PN exposure and can possibly be used as a model for PN allergy in the future. The project described was supported by grants from the National Institute for General Medical Science (GM103427 & 1U54GM115458).

960T **Evaluating Mitochondrial Transport in CHCHD10^{S59L}-Mediated ALS-FTD** Madeleine Chalmers, Qasim Mujteba, Nam Chul KimDepartment of Pharmacy Practice and Pharmaceutical Sciences, College of Pharmacy, University of Minnesota

A single amino acid substitution mutation in the mitochondrial protein, coil-coiled-helix-coil-coiled domain containing 10 (CHCHD10^{S59L}), leads to familial amyotrophic lateral sclerosis with frontotemporal dementia (ALS-FTD). Patients diagnosed with ALS-FTD have motor, behavioral, and cognitive deficits. Axonal mitochondrial transport has been increasingly looked at as a possible contributing factor for neurodegeneration including ALS-FTD. It has been demonstrated that defective mitochondrial transport is present in ALS-FTD disease models, thus reduced mitochondrial transport may play a role in the pathogenesis of ALS-FTD. Previously, we demonstrated that the CHCHD10^{S59L} mutation resulted in abnormal mitochondria morphology and function in *Drosophila* and various cell culture models. In addition, the mitochondrial defects were rescued when the PINK1/Parkin pathway was inhibited by various methods. Although the PINK1/Parkin pathway is well known for maintaining healthy mitochondria as part of the mitochondrial quality control system, it is also very important for mitochondrial transport through neuronal axons.

Therefore, we are evaluating mitochondrial transport in CHCHD10^{S59L}-mediated ALS-FTD disease models. Here, we present live mitochondrial imaging data from *Drosophila* motor neuron and human neuron-like cells to determine how PINK1 dependent mitochondrial movement plays a role in the pathogenesis of CHCHD10^{S59L}-mediated ALS-FTD. We hypothesized that the CHCHD10^{S59L} mutation leads to abnormal mitochondrial movement via PINK1 and reducing

the expression or activity of PINK1 may rescue the mitochondrial transport defects displayed by CHCHD10^{S59L} neurons. This work will define the relationship between CHCHD10^{S59L} and PINK1 on mitochondrial transport, which may lead to therapeutic strategies modulating mitochondrial transport via PINK1 in CHCHD10^{S59L}-mediated ALS-FTD.

961T **Phenylbutyrate modulates polyamine acetylase and ameliorates Snyder-Robinson syndrome in a *Drosophila* model and patient cells** Xianzun Tao[1], Yi Zhu[1], Zoraida Diaz-Perez[1], Seok-Ho Yu[2], Jackson R Foley[3], Tracy Murray Stewart[3], Robert A Casero[3], Richard Steet[2], Grace Zhai[11]University of Miami Miller School of Medicine, [2]Greenwood Genetic Center, [3]The Johns Hopkins School of Medicine

Polyamine dysregulation plays key roles in a broad range of human diseases from cancer to neurodegeneration. Snyder-Robinson syndrome (SRS) is the first known genetic disorder of the polyamine pathway, caused by X-linked recessive loss-of-function mutations in spermine synthase. In the *Drosophila* SRS model, altered spermidine/spermine balance has been associated with increased generation of ROS and aldehydes, consistent with elevated spermidine catabolism. These toxic byproducts cause mitochondrial and lysosomal dysfunction, which are also observed in cells from SRS patients. No efficient therapy is available. We explored the biochemical mechanism and discovered acetyl-CoA reduction and altered protein acetylation as potentially novel pathomechanisms of SRS. We repurposed the FDA-approved drug phenylbutyrate (PBA) to treat SRS using an in vivo *Drosophila* model and patient fibroblast cell models. PBA treatment significantly restored the function of mitochondria and autolysosomes and extended life span in vivo in the *Drosophila* SRS model. Treating fibroblasts of patients with SRS with PBA ameliorated autolysosome dysfunction. We further explored the mechanism of drug action and found that PBA downregulates the first and rate-limiting spermidine catabolic enzyme spermidine/spermine N1-acetyltransferase 1 (SAT1), reduces the production of toxic metabolites, and inhibits the reduction of the substrate acetyl-CoA. Taken together, we revealed PBA as a potential modulator of SAT1 and acetyl-CoA levels and propose PBA as a therapy for SRS and potentially other polyamine dysregulation–related diseases.

962T **A drug repurposing screen to identify therapies for the rare disease DPAGT1-CDG** Hans M. Dalton, Alexys R. Berman, Heather D. Evans, Clement Y. ChowHuman Genetics, University of Utah

Glycosylation encompasses a wide class of biological pathways involving co- and post-translational sugar modifications. Mutations in glycosylation genes underlie Congenital Disorders of Glycosylation (CDGs) – ultra-rare disorders that can cause seizures, developmental delay, and early death. There are few treatment options available for CDGs, and small patient populations make clinical trials difficult. Thus, there is a great need for alternative approaches to finding treatments for these rare diseases. One such alternative is the use of drug repurposing screens that utilize libraries of small molecules with established safety profiles in humans, allowing for potentially faster turnaround for patients.

DPAGT1-CDG is caused by mutations in the gene *DPAGT1*, which encodes the essential first enzyme for N-linked glycosylation. I created a model of DPAGT1-CDG in *Drosophila* using RNAi against *DPAGT1* in the fly eye to cause a small, rough eye phenotype. Using this model, I can assay for drugs that rescue this phenotype by quantitatively measuring its eye size. To find such therapies, I am performing a repurposing screen using 1,520 small molecules that are 98% FDA/EMA-approved (Prestwick Chemical Library). Drugs are mixed into fly food, flies are exposed until early adulthood, and eye size is compared to control flies. The top candidate drugs that rescue, resulting in a larger eye, are later validated by dose-response or genetic analyses.

I currently have a 2.5% hit rate for drugs capable of rescuing the DPAGT1-CDG model eye size ($Z \geq 1.5$). My top candidate drug is a partial NMDA receptor agonist (+45% eye size, Z=2.85), which is important for excitatory neurotransmission. Another top candidate drug is a loop diuretic (+25% eye size, Z=2.04), which inhibits an ion cotransporter. As a proof of principle, I genetically validated this diuretic by using RNAi against its known target gene, and this also increased eye size (+12%). Both hits suggest that ion flux is important in *DPAGT1* deficiency and that this screen can identify new drugs capable of rescuing this disorder. I will present the completed screen of 1,520 drugs, data on candidate drugs and their validation, and overlapping mechanisms of action from the full screen. These drugs may represent novel therapeutic options for DPAGT1-CDG.

963T **Effects of cell-specific expression and deficiency of glucocerebrosidase on sleep in a *Drosophila melanogaster* model of Parkinson's disease** Marissa A Williams[1], Anastasia M Miller[2], Melissa Innerst[2], Kathryn A Jewett[11]Biology, Juniata College, [2]Computer Science and Mathematics, Juniata College

Parkinson's disease (PD) is a common neurodegenerative disorder with many genetic and environmental triggers characterized by motor deficits, memory and behavioral symptoms, and abnormal protein aggregation. We used

a *Drosophila melanogaster* model of PD with a deletion of the gene *Gba1b* which encodes for glucocerebrosidase (GBA). GBA functions as a lipid-modifying enzyme in the lysosome. Without GBA, lipids can accumulate and disrupt lysosomal/endosomal functioning. Flies lacking GBA have movement defects, neurodegeneration, a shortened lifespan, and increased protein aggregation similar to human symptoms of PD. Previous work has shown that some of these phenotypes can be rescued by cell-specific expression of *Gba1b*.

Glial cells are important mediators of intercellular communication and neuronal activity in the brain. Glial GBA has been shown to regulate the lysosomal activity of neurons to influence circadian circuit plasticity and sleep. In humans, PD has been shown to cause disruptions in sleep such as insomnia (decreased sleep at night) and hypersomnia (increased daytime sleepiness). Some *Drosophila* models of PD have found disturbances in their sleep as well. A study using a Minos insertion to disrupt the *Gba1b* gene in flies found them to have significantly less overall time asleep and shorter bouts of sleep compared to wild-type flies. We hypothesize that there will be a significant difference in activity and sleep between control and our GBA-deficient flies. Because glial cells are involved in the regulation of circadian rhythms and sleep, glial-*Gba1b* expression should also alter sleep phenotypes.

In our initial experiments we sought to compare average activity counts in GBA-deficient and revertant control flies. Activity counts were monitored using the TriKinetics Drosophila Activity Monitoring System over a period of 5-7 days using a standard light-dark cycle at 25 degrees Celsius. Our preliminary analysis shows significantly less activity during the light period for GBA-deficient flies, which could correlate to the pathology of PD and its tendency to cause hypersomnia. We plan to examine glial cell-specific expression of *Gba1b* to see if this will affect the changes in activity and sleep observed in GBA-deficient flies.

964T **Characterizing C2C10H^{S81L} (CHCHD10^{S59L}) knock-in *Drosophila* as a model of amyotrophic lateral sclerosis and frontotemporal dementia** Tate J Madson[1], Nam C Kim[1], Anna Gross[21]Pharmacy, University of Minnesota Duluth, [2]University of Minnesota Duluth

Coiled-coil-helix-coiled-coil-helix domain containing 10 (*CHCHD10*) mutations result in dominantly inherited amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD) and related diseases in humans. To investigate CHCHD10^{S59L}-induced disease pathogenesis, we introduced a missense S81L mutation into the *Drosophila* homolog of human CHCHD10, C2C10H gene, using the CRISPR/Cas9-mediated scarless gene editing strategy. The homozygote C2C10H^{S81L} animals exhibited shortened lifespans and progressive motor function defects, including negative geotaxis and flight, in both males and females. Interestingly, the homozygote -inserted knock-out animals showed a female-specific lifespan increase compared to wild-type host animals. We are currently analyzing the effects of heterozygous C2C10H^{S81L} mutation in their lifespan and movement.

965T **Robinow Syndrome *DVL1* mutations cause an imbalance in Wnt signaling pathways during development** Katja MacCharles, Gamze Akarsu, Esther M. VerheyenMolecular Biology and Biochemistry, Simon Fraser University

Insights into how development is regulated by interconnected signaling networks can be gained from studies on developmental diseases. Deciphering the effects of mutations that result in abnormal development through these networks can be difficult due to their complexity. Since flies have little genetic redundancy and are substantially easier, cheaper, and faster to grow than other vertebrate models, using *Drosophila* can help solve the challenge of investigating human disorders. In this work we are characterizing the effects of three distinct *Dishevelled 1* (*DVL1)*variants found in Robinow Syndrome (RS) patients. RS is a rare developmental disorder characterized by shortening of the long bones in the arms and legs and fetal facies like broad nasal tips and widely spaced eyes. Robinow Syndrome has been linked to mutations in several genes which are components of the non-canonical/Planar Cell Polarity (PCP) pathway of Wnt/Wg signaling. Wnt signaling plays a role in both tissue homeostasis and development. DVL proteins are relay molecules for both canonical and non-canonical/PCP Wnt signaling cascades. PCP signaling regulates cytoskeletal processes and directs cell polarity within the epithelial plane. In vertebrates there are 3 DVL proteins while *Drosophila* has a common ortholog, Dsh. The DVL1 patient variants we study have distinct frameshift mutations that cause the C-terminus to be replaced by the same long novel peptide sequence with no known homology. We have used the GAL4-UAS system to express human wildtype DVL1, and the variants found in patients. Our studies have shown that these DVL1 patient variations ectopically stimulate PCP/JNK signaling, trigger apoptosis, and interfere with the stability of Armadillo/b-catenin, thus inhibiting canonical Wnt signaling. We are determining if the DVL1 variants disrupt localization of conserved PCP proteins. These are the first mechanistic studies to show that mutations underlying Robinow Syndrome cause and imbalance in Wnt signaling pathways. Additionally, the mutations cause a number of novel phenotypes in larval and adult tissues, including

anomalies in the anterior cross vein, ectopic bristles, vein thickening and notum abnormalities. These phenotypes suggest DVL1 variants may affect additional conserved signaling pathways.

966T **Tumor invasion initiates at Invasion Hotspots, an epithelial tissue-intrinsic microenvironment** Rei Kobayashi, Junki Ikeguchi, Sheng Deng, Yasuyuki Fujita, Yoichiro TamoriGraduate School of Medicine, Kyoto University

Carcinogenesis is understood as a stochastic process in which mutations in multiple genes, including oncogenes and tumor suppressor genes, accumulate over time, causing what was originally a normal cell to acquire new characteristics and evolve into a cancer cell. Our tumor model in *Drosophila* wing imaginal epithelia, however, shows that not only contingent accumulation of genetic mutations but also the tissue-intrinsic local structures play a key role in the carcinogenic process. We show that genetically mosaic clones of cells mutant for a neoplastic-tumor-suppressor gene in combination with the oncogenic Ras expression initiate invasion into the basal side of the epithelial layer at specific spots in the epithelial tissue. Through the ultrastructural analyses using particle image velocimetry (PIV) and serial block-face scanning electron microscopy (SBF-SEM), we found the patterns of planar-polarized cellular arrangement and the epithelial tissue organization are intrinsically disturbed at the “invasion hotspots.” In addition, the epithelial apicobasal polarity is mildly compromised in these spots. Our genetic experiments show that this local tissue disorganization is further enhanced by the oncogenic mutations, which results in basal mislocalization of the TNF receptor Grindelwald and following JNK-MMP1 signaling activation specifically at the invasion hotspots. Conversely, in other regions of the epithelial tissue, the oncogenic mutant clones do not strongly activate JNK-MMP1 signaling, deviate from the apical side of the epithelial layer, and show benign tumor growth in the lumen. These data indicate that the onset of tumor invasion is highly dependent on the tissue-intrinsic local structure that is structurally vulnerable to oncogenic stimuli. Given the evolutionary conservation of genetic signaling pathways and epithelial architectures involved in this process, tumor invasion from invasion hotspots in Drosophila imaginal epithelia could help us to understand the inevitable factors in carcinogenesis.

967T **A role for the phospholipid transport protein Vps13 in neuronal mitophagy** Ryan Insolera[1], Lynsey Randolph[2], Zachary Haupt[1], Emily Rozich[1], Hubert Osei Acheampong[11]OVAS, Wayne State University School of Medicine, [2]MCDB, University of Michigan

The Vps13-proteins are a family of phospholipid transporters that localize to inter-organelle contact sites. Mutations in all four human Vps13 proteins (VPS13A-D) are associated with neurological disorders, emphasizing the importance of this particular protein family in the maintenance of neuronal health. However, there is currently little understanding of the cellular function of the vps13-proteins in neurons. The *Drosophila* gene *vps13* is the homolog of the two most similar mammalian Vps13 proteins: VPS13A and VPS13C. Mutations in VPS13A are associated with the neurodegenerative disease chorea acanthocytosis, and mutations in VPS13C are associated with a familial form of Parkinson’s Disease. Previously described *vps13* mutant flies recapitulate aspects of the human disorders, exhibiting early lethality and age-dependent neurodegeneration. Here, we describe a critical role for *vps13* in neuronal mitophagy. We find that in *vps13* mutant neurons, mitochondria targeted for degradation via mitophagy initiate, but fail to complete mitophagy as evidenced by the the accumulation of mitochondria that are polyubiquitinated and contain partial phagophores. We term these “stalled mitophagy intermediates”, and posit that their appearance in *vps13* mutant neurons is caused by a defect in phagophore elongation during mitophagy, interrupting autophagosome formation and delivery of damaged mitochondria to the lysosome for degradation. In conditions of genetic- or pharmacologically-induced mitochondrial stress, these stalled mitophagy intermediates become more abundant in *vps13* mutant fly brains, and correlate with increased lethality. We observe that endogenous Vps13 protein localizes to puncta closely apposed to mitochondria, including polyubiquitinated mitochondria that are actively undergoing mitophagy. These results suggest that vps13 plays a critical role in efficient neuronal mitophagy, likely facilitating the transfer of phospholipids to the growing phagophore on mitochondria during mitophagy. Finally, this work indicates that defective mitophagy may be a contributing factor to the human neurological disorders associated with mutations in VPS13A and VPS13C.

968T **Screening for Genetic Modifiers of *MED12/kto* Using Naturally Occurring Variation in *Drosophila melanogaster***
Kristin C Bussey, Nestor O.N. Yepiz, Vijay Shankar, Robert R.H. Anholt, Trudy F.C. MackayGenetics and Biochemistry, Clemson University

MED12 of the Mediator Complex is associated with five rare genetic disorders characterized by intellectual disability, behavioral, and congenital defects. Different individuals with the same MED12 mutation present differing severity

of symptoms, suggesting the presence of genetic modifiers. However, rarity and diversity of these disorders make identifying the genetic basis of pathogenicity and genetic modifiers challenging in human populations. *Drosophila melanogaster* has a strongly conserved functional ortholog of MED12, *kohtalo (kto)*. I am taking advantage of natural variation among fully sequenced inbred lines of the *Drosophila melanogaster* Genetic Reference Panel (DGRP) to identify genetic modifiers altering effects of *kto* mutations. Preliminary data identified two quantitative behavioral phenotypes, locomotion and sleep/activity, as ideal screening phenotypes in *D. melanogaster*. To identify modifier loci, I cross a null mutation of *kto* (kto^{Δ}), and a wild type *kto* allele (kto^{+}) to the DGRP lines. I quantify sleep behavior and startle-induced locomotion in the F_1 offspring. Over 90 (60) genetic backgrounds have thus far been tested for startle-induced locomotion (sleep and activity) and show significant DGRP line by *kto* genotype interactions for startle response and sleep traits, with both amelioration and exacerbation of effects in kto^{Δ} relative to kto^{+} heterozygous genotypes. I will screen 200 DGRP lines crossed to kto^{Δ} and kto^{+} for startle response and sleep and activity traits. Upon completion of the modifier screen, I will perform genome wide association analyses on the difference in phenotypes between kto^{Δ} and kto^{+} F_1 heterozygotes for each DGRP line to identify candidate genetic modifiers of *kto*, generate a genetic interaction network to superimpose human orthologs upon, and perform functional tests of these modifier variants with DGRP lines not included in the initial screen. Identification of top modifier candidates will provide insight into how genetic modifiers may influence the manifestation and severity of MED12-related disorders.

969T **A genetic screen for model of PIGA deficiency identifies *GSK3B* as a candidate modifier** Miriam C Aziz, Shayna N Scott, Emerald Lane, Emily Coelho, Clement Y ChowHuman Genetics, University of Utah

Phosphatidylinositol glycan class A (*PIGA*) deficiency is an X-linked congenital disorder of glycosylation (CDG). CDGs are rare genetic diseases caused by mutations in genes that affect the biosynthesis or addition of glycans to other macromolecules. PIGA deficiency shares many symptoms with other CDGs such as shortened lifespan, hypotonia, facial dysmorphism, and epileptic seizures. *PIGA* encodes an enzyme that catalyzes the first step of glycosylphosphatidylinositol (GPI) biosynthesis. PIGA transfers a N-acetylglucosamine onto phosphatidylinositol, forming the first intermediate of the pathway. GPI-anchored proteins (GPI-APs) are localized to the cell surface and involved in processes such as endocytosis, immunity, and signal transduction. Like many CDGs, variability in phenotypic severity is common in PIGA deficiency patients, though the reason behind this variability is unknown. Because these disorders are often congenital, background genetics likely plays a big role. It is necessary to study many genetic backgrounds to fully understand what affects the phenotypic outcome of a disease. To do this, we performed a genetic screen that utilizes the genetic variation found in ~200 lines of the *Drosophila* Genetic Reference Panel (DGRP) to identify potential genetic modifiers that impact seizure susceptibility in our PIGA deficient *Drosophila* model. We found that seizure susceptibility and median seizure recovery time associated with PIGA deficiency are both highly dependent on genetic background. We observed a moderate correlation between seizure susceptibility and recovery time, suggesting only a partial overlap between the genetic architecture that underlies variability of either phenotype. To identify modifiers of these phenotypes, we performed genome wide association analyses for each independently and by sex. This screen identified *Glycogen Synthase Kinase 3B* (*GSK3B,* or *sgg* in *Drosophila*) as a top candidate modifier of PIGA deficiency. *GSK3B* is ubiquitously expressed, but highly enriched in the brain. GSK3B is involved in multiple signaling pathways such as Wnt and insulin signaling, and plays a role in microtubule regulation, neuronal development, axon outgrowth, and more. Dysregulation of any of these processes may prime the phenotypic severity seen in *PIGA* deficient patients. We will present characterization of GSK3B as a modifier using pharmacological and genetic approaches. Further inquiry into GSK3B as a genetic modifier of PIGA deficiency will provide insights into improved treatments and personalized therapies.

970T **Using *Drosophila* to design optimized therapeutic exercise programs across neurodegenerative diseases** Alyson Sujkowski[1], Nadir Alam[1], Sokol V Todi[1,1,21]Pharmacology, Wayne State University, [2]Neurology, Wayne State University

Recently, the need to extend long-term health, promote independence, and protect against diseases has become a critical aim intended to reduce rising healthcare burdens in aging populations across the globe. One of the most widely studied, low-cost, and largely accessible interventions that promotes these 3 interrelated healthcare needs is endurance exercise. Exercise not only improves health and enhances independence, but also protects against a variety of disorders like metabolic syndrome, cardiovascular disease, cancer, and neurological dysfunction. In the nervous system, exercise supports neural plasticity to enhance memory, improve mood and to prevent neurodegeneration, but following a prescribed exercise program becomes more difficult as disease symptoms progress. Clinical studies indicate that varying exercise intensity may be one way to delay disease progression without worsening neurodegeneration. Unfortunately, the optimal exercise regimen and critical treatment window is difficult to identify across individuals and diseases. In

order to circumvent these problems, I used our *Drosophila* endurance exercise paradigm in conjunction with our lab's isogenic neurodegenerative disease model flies. Both systems have demonstrated success identifying both conserved adaptations and disease-modifying pathways that parallel those in higher organisms, including rodents and humans. My preliminary observations indicate age-, tissue- and disease-specific effects on survival and motility that depend on exercise regimen and timing. Current work aimed at identifying the molecular mechanisms underlying these benefits will be leveraged to design disease- and age-targeted programs that may be used in the future to 1) increase exercise accessibility to a wide range of patients and 2) identify exercise-mimicking factors that may be able to improve quality of life for individuals no longer able to perform an exercise program as prescribed.

971T **The 21 bp deletion mutant calpain3 does not inhibit wild type calpain3 activity.** Seung-Jae Oh, Yun-Jeong Choe, JiHye Kim, Swati Maitra, Nam Chul KimUniversity of Minnesota

Loss-of-function mutations in the Calpain3 gene have been related to autosomal recessive *Limb-Girdle Muscular Dystrophy-1* (LGMDR1), a common form of muscular dystrophy. Calpain3 is one of the calpain protease family member that is a calcium dependently regulated proteolytic enzyme mainly expressed in skeletal muscle. Although LGMDR1 is an autosomal recessive disorder caused by homozygous Calpain3 loss-of function mutations or compound heterozygote mutations, interestingly, autosomal dominant calpainopathy with a heterozygous 21-bp deletion in Calpain3 gene (*Autosomal Dominant Limb-Girdle Muscular Dystrophy-4, LGMDD4*) has been reported and suggested that mutant proteins act in a dominant-negative manner suppressing the activity of wild type Calpain3. Therefore, we have examined whether the deletion mutant form of Calpain3 has a dominant-negative activity against its wild type of counterpart using human cells and in vivo *Drosophila* models. Here, we present data showing that the deletion mutant Calpain3 does not suppress wild type Calpain3 activity. Rather, it is a mutant lacking autocatalytic processing activity like many other loss-of-function Calpain3 mutants. Our results implicate that more thorough investigation is necessary to understand the dominant inheritance of the heterozygous 21-bp deletion mutation in LGMDD4.

972F **Differential Impacts of Manipulated Microbiome Composition of Natural Diets on *Drosophila's* Fitness and Metabolic Phenotypes** oluwatobi E fijabi[1], Derek E Maas[1], laura Reed[21]Biological Sciences, University of Alabama, [2]University of Alabama

The interaction between diet and microbiome influences metabolism. We know that lab diets influence gut ecosystem differently from natural diets. Here we assess the influence of two rotten peach and strawberry on metabolic traits of *Drosophila.* We hypothesize that manipulating the microbiota content of rotten fruit will induce genotype-by-diet interactions on larval traits. To test this, we eliminated maternal bacteria from embryos of three genotypes, then raised them on the rotten fruit diets. We also eliminated microbes in these diets with antibiotics and autoclaving. Antibiotics don't remove all microbes, while autoclaving does, however, autoclaving can cause nutrient degradation.

To determine the microbiome communities and nutrient within the diets, diet samples were subjected to 16S sequencing, proximate analysis, and untargeted metabolomic profiling (GC/MS). We found that *Gluconobacter oxydan* increased in incubated peach, the concentration of carbohydrate and soluble sugars reduced upon incubation for the peach diet, especially trehalose sugar. The alpha-diversity of microbiome was higher in strawberry ($P<0.005$).

We assessed survival, development to 3rd instar, weight, triglyceride (TAG), and total protein (TP) content of larvae fed rotten diets. Strawberry fed larvae had greater survival and shorter development times ($P<0.001$). Autoclaving reduced survival on both fruits and there was higher survival in antibiotic treated strawberry compared to antibiotic treated peach. Larvae on autoclaved diets had the longest development time, while antibiotic treatments had shorter, and untreated had the shortest. Autoclaved strawberry fed larvae had reduced weight compared to peach autoclaved, while other diet-treatment combinations resulted in higher weights. Genotype influenced the total protein of larvae. A diet-by-treatment interaction was observed for TAG, with increased levels in autoclaved strawberry fed larvae, while a genotype-by-treatment interaction was observed for TP, and larvae fed manipulated diets had reduced TAG concentration. Thus, an autoclaved diet reflects a stressful environment for larvae growth. Strawberry has higher nutritional and microbial content, and a better substrate for *Drosophila*. Elimination of dietary microbes explained the highest variance observed for traits and induced a diet-by-genotype interaction.

973F **Cooperated regulation of Notch and Yki promotes Mmp1 production in transition-zone tumorigenesis** Chun-Ming Jimmy Lai, Wu-Min DengBiochemistry and Molecular Biology, Tulane University School of Medicine

Transition zones (TZs) are regions in the animal body where two types of epithelial tissue meet. Many TZs are prone to

tumorigenesis, probably because of their less-defined cell fate and conflicting signals received from both sides of the epithelial tissues. However, the underlying mechanism of tumor susceptibility in the TZs remains largely unclear. We have recently reported a tumor model in the *Drosophila* larval salivary gland imaginal ring (ImR), were upregulated Notch signaling drives neoplastic tumor growth through the overexpression of Notch intracellular domain (NICD), at a narrow epithelial TZ that resides at the posterior end of the ImR and borders the secretary polytene salivary gland cells. These NICD-TZ tumor cells lose cell polarity and upregulate Mmp1 via JNK signaling, which results in basal delamination and metastasis in the advanced tumors. Beside the posterior end of SG ImR, we also identified several other TZs, including in the anterior part of SG ImR, the foregut and hindgut ImRs. Interestingly, we found that different TZs display different tumor susceptibility upon Notch hyperactivation. We hypothesize that they might be distinct in either epithelial identity or accessibility of tumor promoting factors. Consistent with latter hypothesis, knockdown of Hippo or overexpression of a constitutively active form of Yki promotes NICD-induced tumorigenesis in all TZs. Intriguingly, we found that Notch hyperactivation enhances both secreted and membrane-tethered forms of Mmp1 in the posterior SG TZ, while only increases secreted form of Mmp1 in the anterior SG, foregut and hindgut TZs. However, co-overexpression of Yki and NICD promotes both secreted and membrane-tethered forms of Mmp1 in all TZs, suggesting that Notch and Yki synergistically promote TZ tumorigenesis through regulation of different Mmp1 isoforms. Mmp1 promotes tumor growth and metastasis by interacting tumor growth factors and degrading extracellular matrix (ECM) via different isoforms, thus it is critical to decipher the regulation of Mmp1 isoforms in tumorigenesis. Currently, we are investigating the underlying mechanism of how Notch and Yki differentially regulate the production of Mmp1 isoforms in different TZs through transcriptomic analysis.

974F **Metabolic Disruptions Link Fragile X Syndrome and Glycogen Storage Disease Type IX** Aashi R Gurijala, Emma Rushton, Kendal S BroadieBiological Sciences, Vanderbilt University

The *Drosophila* Fragile X syndrome (FXS) model is well established, with proven conservation to the human disease state. A possible relationship between FXS and Glycogen Storage Disease Type IX (GSD) surfaced in a 1993 case study on “patient 2”, with mutations in the Phosphorylase Kinase Regulatory Subunit Alpha 2 (*PHKA2*) as well as the Fragile X Mental Retardation Protein (FMRP) KH-type RNA-binding domain causing symptoms far more severe than either disease alone, including heightened intellectual disability, extreme macroorchidism, physical deformations, and difficulties with communication and movement. We hypothesized interaction between FXS and GSD disease states results in unsustainably elevated metabolic demand due to known heightened glycolysis metabolism in FXS. There was no established *Drosophila* GSD model, but *PHKA2* mutant and RNAi lines are both available. We first assayed third instar neuromuscular junction (NMJ) synaptic architecture defects, a well-established FXS phenotype, to compare mutant genotypes. We find significant differences in synaptic branch length and bouton number in both *dfmr1* and *PHKA2* mutants, as well as double heterozygote animals. We next investigated GSD effects on larval metabolism, using MitoTracker Orange™ to assay mitochondrial changes compared to genetic background controls. After starving third instars for 15 hours, we saw a significant decrease in mitochondrial fluorescence between fed and starved control larvae. In contrast, starving *PHKA2* RNAi larvae caused no effect on mitochondria, with both conditions comparable to starved controls. Driving *PHKA2*^{EPG104} over-expression does not significantly alter mitochondria, indicating *PHKA2* loss causes the metabolic phenotype. Furthermore, regions of spatially absent mitochondria are found in muscles of both FMRP and *PHKA2* loss-of-function animals. We observed that double RNAi animals have a significantly elevated number of mitochondria-deficient regions of a greater average size. Greater disruptions in mitochondria distribution in double RNAi animals relative to both single RNAi larvae indicate a detrimental metabolic interaction. We are continuing to test the metabolic demand interaction between FXS and GSD disease models with a range of newer transgenic reporters, and assaying consequent phenotype intersections within the neuromusculature.

975F **PolyQ Expansion and traumatic brain injury (TBI) cause mitochondria dysfunction via distinct mechanisms** Kelsey Swinter[1], Dania Salah[2], Shermali Gunawardena[11]Biological Sciences, University at Buffalo, [2]University at Buffalo

Mitochondrial dysfunction is observed in several Huntington’s disease (HD) models. However, it is unclear how mitochondrial defects occur during HD neuropathogenesis. Here we hypothesize that excess pathogenic huntingtin (HTT) impairs mitochondrial homeostasis. We observe that pathogenic HTT show fragmented mitochondria in addition to axonal blockages and cell death compared to normal HTT. Expression of pathogenic polyQ alone or in the context of another polyQ expansion disorder (MJD/SCA3) also caused mitochondrial fragmentation in contract to expression of non-pathogenic polyQ repeats, indicating that mitochondrial defects are not specific to loss/gain of HTT function.

Clearance of polyQ accumulations by excess chaperone protein HSC70 rescued mitochondria fragmentation, suggesting that polyQ-mediated fragmentation is likely due to polyQ accumulation. However, rescue of polyQ-mediated cell death via activation of the PI3K pathway failed to rescue mitochondria fragmentation, suggesting that polyQ-mediated fragmentation is not the result of cell death, further supporting the prediction that mitochondrial defects are a consequence of polyQ aggregation. Expression of excess fusion protein mitofusion (MFN) or depletion of the fission protein dynamin-related protein 1 (DRP1) rescued PolyQ-mediated mitochondria fragmentation, suggesting that changes to the balance of mitochondrial fission-fusion can contribute to polyQ-mediated fragmentation. Further inhibition of NOS rescued polyQ-mediated fragments, suggesting that perhaps excess nitrosylation of DRP1 causes polyQ-mediated fragments. However, polyQ-mediated mitochondria fragments did not show evidence of oxidative damage, but were depolarized with decreased calcium storage. Taken together our observations propose a model in which pathogenic polyQ aggregations increase NO production leading to excess DRP1-nitrosylation mediated mitochondrial defects. Alternatively, mitochondrial defects could occur due to a direct response of stress placed on neurons by pathogenic polyQ aggregations. While mechanical stress induced by traumatic brain injury (TBI) caused axonal transport defects, neuronal cell death, and mitochondria fragmentation, activation of the PI3K pathway rescued mitochondria fragmentation, indicating that TBI-mediated mitochondrial fragmentation is likely the result of cell death. Taken together our observations unravel distinct mechanisms for mitochondrial defects in expanded polyQ disorders and TBI.

976F **The Human Antimicrobial Peptide, LL-37, Mitigates Aβ's Effects on Gene Expression in a Drosophila Model of Alzheimer's Disease** Marissa E Joe[1], Joseph Lyons[1], Kenneth Owyang[1], Sahar Sabaghian[1], Anisha Kavarthapu[1], Belal Alatasi[1], MaiLan Kasch[1], Ruby Guevara[1], Jennifer Tan[1], Taylor Jones[1], Annelise Barron[2], Jeremy Lee[11]Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, [2]Bioengineering, Stanford University

Alzheimer's disease (AD) is characterized by the presence of amyloid plaques in affected areas of the brain. These aggregates are composed largely of the amyloid beta (Aβ) peptide, which has been shown to be neurotoxic and an important contributor to AD pathology. While the function and effects of Aβ are not well understood, recent studies have shown it may have antimicrobial effects (Kumar et al., 2016). The human antimicrobial peptide LL-37 has been shown to bind Aβ in vitro. Furthermore, LL-37 binding induced a decrease in aggregation of Aβ in vitro (De Lorenzi, et al., 2017). If LL-37 and Aβ interact in vivo, it may affect Aβ aggregation and, as a result, its neurotoxicity.

We previously generated four transgenic fly lines that express the human peptides Aβ and LL-37 pan-neuronally using the Gal4-UAS system: LL37-expressing, Aβ and LL-37 co-expressing, Aβ-expressing, and non-expressing control flies. Longevity and RING climbing assays have shown that co-expression of LL-37 and Aβ in flies led to longer lifespans than flies expressing only Aβ, but had shorter lifespans than the non-expressing control flies. Consistently, co-expressing flies showed a significant improvement over Aβ expressing flies in both survivorship to eclosion and RING assays. These results indicate that LL-37 partially attenuates the deleterious effects of Aβ in vivo.

Recently we compared the expression profiles of our experimental transgenic flies, using RNA sequencing. These analyses show that the misregulation of proteolytic genes in Aβ-expressing flies is at least partially alleviated by the co-expression of LL-37. This was ascertained by finding the overlapping genes between those that were upregulated by Aβ and subsequently downregulated by co-expression of LL-37 and vice versa. This provides one possible mechanism by which LL-37 alleviates the deleterious effects of Aβ.

To determine whether Aβ and LL-37 directly interact or are components of the same complex in vivo, we are carrying out co-immunoprecipitation (co-IP) experiments using our LL37/Aβ co-expressing flies. If the co-IP results indicate that Aβ and LL-37 are in fact components of the same complex in vivo, then this would further suggest that LL-37, and/or factors that increase its expression, could be useful targets for developing future therapeutics for Alzheimer's disease.

977F ***Drosophila*** **nutrigenomics to identify diets that treat inherited amino acid disorders** Jiayi Lin[1], Felipe Martelli[2], Sarah Mele[2], Oguz Kanca[3], Chris Barlow[2], Ralf Schittenhelm[2], John Christodoulou[4], Hugo J. Bellen[3], Matthew D.W. Piper[2], Travis K. Johnson[21]School of Biological Sciences, Monash University, [2]Monash University, [3]Baylor College of Medicine, [4]Murdoch Children's Research Institute

Amino acid disorders (AADs) are a group of over 1,000 highly heterogeneous genetic diseases that individually are rare, but collectively impact approximately one in every 6,500 babies born. Many AADs lead to severe neurological debilitation and death within the first few weeks of life, but in a few instances, dietary therapy has proven to have remarkable effects, preventing major neurological damage and allowing individuals to live normally. However, conceiving and trialling new diets in the clinic is made difficult because of the low birth incidence and often early onset of these

diseases. Our team is addressing this by establishing a scalable *Drosophila*-based pipeline that incorporates nutrient-disease interactions and metabolomic profiling to find new diets that may be of clinical value. So far, we have generated over 40 distinct AAD fly models. These models recapitulate clinically relevant impairments in development, neurological and metabolic function, and mirror relative differences in patient disease severity and age of onset. By applying rapid high-throughput diet screening that covaries several nutrients simultaneously, we have been able to identify nutrient formulations that ameliorate symptoms and restore lifelong fly health (e.g. for isolated sulfite oxidase deficiency, a sulfur-amino acid catabolism disorder). These models and diets can further be used to define necessary treatment windows and uncover mechanisms of disease pathogenesis. Our work establishes *Drosophila* as a highly suitable organism for modelling AADs and we hope that our approach will expedite the process of identifying new and effective diets to help reduce the suffering caused by these diseases.

978F **The role of the mitochondrial enzyme Scully in dementia** Maya Solis[1], Carolyne Chepkosgei[2], Paul Sabandal[2], Kyung-An Han[11]The University of Texas at El Paso, [2]Biological Sciences, The University of Texas at El Paso

Dementia etiology is complex involving both genetic and non-genetic components. The genetic and non-genetic risk factor interactions contributing to dementia remain poorly understood. To address this knowledge gap, we conducted a genetic screen to identify novel dementia genes that interact with non-genetic risk factors (e.g., aging and sleep anomaly) using inhibitory control deficit as an endophenotype in *Drosophila*. We identified 13 dementia genes, one of which is Scully. Scully is the homolog of 17-β-hydroxysteroid dehydrogenase 10 encoding a multifunctional mitochondrial enzyme that is known to bind Aβ peptides. We found that the flies with the heterozygous mutation in *Scully* (*Scu/+*) exhibited substantial deficits in inhibitory control and short-term memory in an aging-dependent manner. To determine the underlying mechanism, we examined the mitochondria number (via mCherry.mito reporter) and turnover (via MitoTimer3 reporter) in the mushroom body (MB) neurons of *Scu/+* and *CS* at three different ages (4 days, 2 weeks, and 4 weeks old). We found that *Scu/+* mutants had lower mCherry.mito expression compared to *CS* flies at 4 days old, suggesting that *Scu* may have a role in mitochondria biogenesis. In the mitochondria turnover assessment, we observed no differences in MitoTimer3 maturation between *Scu/+* and *CS* at all ages tested. However, we noticed a significant accumulation of mature mitochondria in both *Scu/+* and *CS* with aging. This suggests that aging impacts mitochondrial turnover and that Scu is not involved in this phenomenon. Currently, we are assessing the role of *Scu* in oxidative stress (e.g., reactive oxygen and reactive nitrogen species) and bioenergetics (e.g., ATP and glycolysis), and the findings of these studies will be presented. Our study will provide novel mechanistic insights by which genetic and non-genetic risk factors interact for dementia.

979F **Neurodegenerative Phenotypes Associated with Mutant tRNA Endonuclease RNase Z in *Drosophila*** Saathvika Rajamani[1], Alessia Vata[1], Max Luf[2], Edward B Dubrovsky[11]Biological Sciences, Fordham University, [2]Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai

Transfer RNA (tRNA) molecules are short non-coding RNAs that play a vital role in decoding genetic information during translation. Biogenesis of functional tRNA occurs through several catalytic reactions. Mutant variants of pre-tRNA processing enzymes have been associated with the development of neurodegenerative disorders in human patients. One of these enzymes is the RNase Z endoribonuclease, encoded by the *ELAC2* gene. Its role is to cleave off a 3' trailer from nascent tRNA. Patients reported with mutations in *ELAC2* displayed several neurological disease symptoms, including neurodevelopmental delay, muscular hypotonia, and microcephaly. Given that this gene is highly conserved among eukaryotes, we decided to study its role in neurodegeneration using the *Drosophila* model. To do so, we employed CRISPR/Cas9 technology to generate different fly lines wherein RNase Z was modified in a neuron-specific manner. In our first model of complete neuronal knockout of RNase Z, we observed pronounced locomotor deficits followed by early death. To determine whether this deficit could be due to motor neuron damage, we analyzed the neuromuscular junctions (NMJ) of the flight muscles, and we indeed observed severely compromised neuronal morphology with marked reductions in branch length and number. Since human patients carry missense rather than knockout alleles, we next created a fly model that expressed one of the RNase Z missense variants associated with neuropathology. Histological analysis of the mutant adult brains showed increased vacuolization, a classical hallmark of neurodegeneration. These flies also exhibited locomotor deficiency evidenced by the decreased climbing index, and significantly reduced neuronal branch length and numbers, suggesting synaptic denervation at the NMJs. Given that RNase Z plays a crucial role in mitochondrial tRNA processing, we hypothesize that the associated neurodegeneration could be due to mitochondrial dysfunction. As a first step in this direction, we analyzed the outcome of knocking out RNase Z in neuronal mitochondria. These flies presented loss of flight ability similar to the neuronal knockout, accompanied by reductions in neuronal branch length and branch number at the NMJs. This result indicates that inactivation of neuronal mitochondrial RNase Z

is sufficient to produce the neuropathological phenotype. Collectively, our findings suggest an important role for RNase Z in maintaining proper neuron function in Drosophila.

980F **Oncogenic stress-induced Netrin reprograms systemic metabolism as a humoral inter-organ molecule in *Drosophila*** Morihiro Okada, Tomomi Takano, Yuko Ikegawa, Hanna Ciesielski, Hiroshi Nishida, Yoo SaKanRIKEN

Cancer exerts pleiotropic, systemic effects on organisms. Health of organisms with cancer deteriorates, eventually leading to organismal death. How cancer induces systemic effects on remote organs and the organism itself still remains elusive. Here we describe a role for NetrinB (NetB), a protein with a particularly well-characterized role as a tissue-level axon guidance cue, in mediating oncogenic stress-induced organismal, metabolic reprogramming as a systemic humoral factor. Ras-induced dysplasia upregulates and secretes NetB. Inhibition of either NetB from the transformed tissue or its receptor in the fat body suppresses oncogenic stress-induced organismal death. Mechanistically, NetB from the dysplastic tissue remotely suppresses carnitine biosynthesis, which is critical for acetyl-CoA generation and systemic metabolism, in the fat body. Supplementation of carnitine or acetyl-CoA inhibits oncogenic stress-induced organismal death. This is the first identification, to our knowledge, of a role for the Netrin molecule, which has been studied extensively for its role within tissues, in humorally mediating systemic effects of local oncogenic stress on remote organs and organismal metabolism.

981F **Insulin signaling activation in the fat body suppresses wing disc tumor growth through regulating lipid metabolism and transfer** Chen Yang, Yang Yang, Jingjing He, Yan YanDivision of Life Science, Hong Kong University of Science and Technology

Drosophila melanogaster has emerged as a powerful model organism for studying interactions between growing tumors and other organs. In *Drosophila* larvae, mutations in *scribble* (*scrib*) and *discs large* (*dlg*), which encode conserved cell polarity genes, lead to malignant tumor growth in wing imaginal discs and optic lobes. While several studies have shown that the *scrib* or *dlg* mutant tumors interact with the fat body, the extent to which tumor-adipocyte signaling affects tumor progression remains to be further explored.

We have previously found that the *scrib* and *dlg* mutant tumors undergo severe growth arrest in the first 7 days after egg laying (AEL), followed by a rapid growth phase into huge tumor masses. We found that the expression of *insulin-like peptide 6* (*ilp6*) is highly up-regulated in the early *scrib* mutant tumors in comparison with wild type wing discs. Moreover, tumor-derived Ilp6 lead to insulin signaling activation in the fat body adjacent to wing discs. Depletion of Ilp6 through RNAi in the tumor releases the early *scrib* or *dlg* mutant tumors from growth arrest. Blocking Insulin signaling activation in the fat body also lead to a release from growth arrest in the early *scrib* or *dlg* mutant tumors. Moreover, if we maintain high insulin activity in the fat body through expressing InR^{CA}, the *scrib* or *dlg* mutant tumors remain in a prolonged growth arrest state until larval death. To dissect the mechanisms of how insulin signaling activity in the fat body regulates tumor growth, we performed quantitative proteomics analysis and found that insulin signaling level in the fat body likely affects lipid metabolism. We identified that Brummer (Bmm), a major lipid storage regulator in the fat body, functions downstream of insulin signaling activation to regulate tumor growth. Overexpression of *Bmm* in the fat body depletes lipid storage in the fat and causes a prolonged growth arrest of the *dlg* mutant tumor. Moreover, we identified that an apolipoprotein Nplp2 in the fat body controls the *dlg* mutant tumor growth. Taken together, these results suggest that insulin signaling activation in the fat body suppresses wing disc tumor growth through regulating lipid metabolism and transfer.

982F **Identification of cyst-reducing molecules to improve polycystic kidney disease** Jay DeLoriea[1], Edenborough Hibionada[1], Michael Monroe[1], Christian Linen[1,2], Eliya Karoutchy[1,3], Amber Wilson[1], Cassandra Millet-Boureima[4], Anh Minh Thao Nguyen[5], Ramesh Chingle[5], William D. Lubell[5], Chiara Gamberi[11]Biology, Coastal Carolina University, [2]Academy of Arts and Sciences, [3]Scholars Academy, [4]Biology, Concordia University, [5]Chemistry, Universite de Montreal

Chemical probing and drug assays in adult *Drosophila* can complement genetic screens by enabling examination of organismal physiology and manipulation of the activity of key cellular regulators without altering the expression of their cognate gene. Drug assays using large fly populations may reveal diseased phenotype-modification potential and are statistically robust. We have investigated drug-testing in *Drosophila* for reduction of renal cysts in a new fly model of polycystic kidney disease (PKD) established in our lab. PKD causes the progressive cystic and neoplastic degeneration of the kidney and kidney failure through complex, largely unknown mechanisms. Alike PKD patients and PKD animal models, PKD model flies respond to rapamycin treatment by transiently reducing cyst number and size. [1] Mimics of the Second mitochondria-derived activator of caspases (Smac) have been used as anti-neoplastics to induce cell death

in several cancers and, in one case, to alleviate cysts in a murine PKD model.[2] We found that four novel Smac mimics produced by chemical synthesis exhibited distinct cyst-reducing potency, corroborating evidence for the drug potential of Smac mimicry in PKD. [3] Further implicating deregulated neoplastic signaling in the cystic fly, melatonin showed cyst-reducing potential and -due to lack of toxicity- also drug potential. [4] We now report new, chemically synthesized, molecules that exhibit 40-80% cyst-reducing activity in vivo. The renal tubule of many organisms, including *Drosophila*, has regional specializations for selective ion and water trafficking to maintain homeostasis. Remarkably, the tested compounds appeared to affect distinct regions of the renal tubule differentially, suggesting potential for chemical manipulation of different tubular regions both experimentally and, potentially, therapeutically. Therefore, *Drosophila* pharmacology can be effectively added to the fly toolbox to tease apart the complex biology of disease.[5] *References*

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983F **Mapping Convergence of Oncogenic Ras, Notch and Yki Signaling in a *Drosophila* Tumor Model** WANG YING, Mingxi DengLife Science, HKUST

The Scribble complex (Scribble/Scrib, Discs-Large/Dlg, and Lethal giant larvae/Lgl) is a highly conserved cell polarity complex important for maintaining epithelial architecture in both *Drosophila* and mammals. These genes were originally discovered in *Drosophila* as “neoplastic tumor suppressor genes” (nTSGs) because fly larvae homozygous mutant for this group of genes carries malignant tumors in imaginal discs and optical lobes. Interestingly, when the *scrib* or *dlg* mutant cells are generated as mosaic clones and surrounded by wild-type cells, these mutant clones fail to survive and undergo apoptosis. The growth outcome of the *scrib* or *dlg* mutant clones can be completely reversed through modulation of Ras, Notch, or Hippo signaling activities. To investigate whether these oncogenic signals converge at critical downstream modules to promote tumorigenesis, we used single-cell transcriptomic techniques to profile wing imaginal discs harboring the $scrib^{-/-}$, $scrib^{-/-}Ras^{V12}$, $scrib^{-/-}NICD$, $scrib^{-/-}Yki^{S168A}$ clones. Our single-cell transcriptomic analysis indicated that Ras, NICD, and Yki signaling do not change the intrinsic capacity of cell proliferation or death in these clones autonomously. Instead, we found that the $scrib^{-/-}Ras^{V12}$, $scrib^{-/-}NICD$ or $scrib^{-/-}Yki^{S168A}$ clones express significantly higher level of *Upd2* in comparison with the $scrib^{-/-}$ clones, which triggers high STAT signaling activation in cells surrounding these clones non-autonomously. Depletion of Upd2 blocks the growth of the $scrib^{-/-}Ras^{V12}$, $scrib^{-/-}NICD$, $scrib^{-/-}Yki^{S168A}$ clones. Non-autonomous STAT activation is necessary to promote the *scrib* mutant clonal growth in these contexts. Together, our data suggest that Ras, NICD, and Yki signals converge to elevate Upd2 expression which non-autonomously activates STAT signaling to promote the *scrib* mutant clone survival.

984F **Investigation of metabolism reprogramming in *Drosophila scribble* mutant tumor model** Mingxi DENGLIFS, Hong Kong University of Science and Technology

Metabolic reprogramming is a cancer hallmark important for supporting aberrant growth. However, the complexity of human cancers limits mechanistic understanding of tumor metabolic rewiring processes. Through analyzing longitudinal bulk and single cell RNA sequencing data for the *scrib* mutant tumors, a classical *Drosophila* tumor model, we found that the *scrib* mutant tumors likely undergo metabolic reprogramming as tumors progressing through different stages. Moreover, the *scrib* mutant tumor cells showed a high degree of heterogeneity in their metabolic signatures. We found that a population of tumor cells with elevated glycolytic activity emerge as these tumors progress to later stages. The glycolytic cell population accelerated glucose uptake by expressing a novel glucose transporter, *MFS3*.We further found that a FGF ligand, *bnl*, which is secreted in this glycolytic cell population, is required for tumor growth.

985F ***Drosophila* harboring epilepsy-associated mutations in the vitamin B6 metabolism gene *PNPO* display allele-specific and diet-dependent excitability phenotypes** Wanhao Chi[1], Atulya Iyengar[2,3], Wenqin Fu[1], Wei Liu[4], Abigayle Berg[3], Chun-Fang Wu[3], Xiaoxi Zhuang[11]Dept. Neurobiology, Univ. Chicago, [2]Dept. Biol. Sci., University of Alabama, [3]Dept. Biology, Univ. Iowa, [4]Envron. Health, China Medical Univ.

Pyridox(am)ine 5’-phosphate oxidase (PNPO), encoded by *sugarlethal* in flies, catalyzes the rate-limiting step in the

synthesis of pyridoxal 5'-phosphate (PLP), the active form of vitamin B6 required for the synthesis of neurotransmitters gamma-aminobutyric acid (GABA) and the monoamines. Pathogenic variants in the human ortholog *PNPO* have been increasingly identified in patients with neonatal epileptic encephalopathy and early-onset epilepsy. These patients often exhibit different types of seizures and variable comorbidities. Recently, the *PNPO* gene has also been implicated in epilepsy in adults. It is unclear how these phenotypic variations are linked to specific *PNPO* alleles and to what degree diet can modify their expression. Using CRISPR-Cas9, we generated four knock-in *Drosophila* alleles, h^{WT}, h^{R116Q}, h^{D33V} , and h^{R95H}, in which the endogenous *Drosophila sugarlethal* gene was replaced by wild-type human *PNPO* complementary DNA (cDNA) or *PNPO* carrying three epilepsy-associated variants. We found that these knock-in flies exhibited a wide range of phenotypes, including developmental impairments, abnormal locomotor activities, spontaneous seizures, and shortened life span. These phenotypes are allele dependent, varying with the known biochemical severity of these mutations and our characterized molecular defects. We also showed that diet treatments further diversified the phenotypes among alleles. Strikingly, PLP supplementation at larval and adult stages prevented developmental impairments and seizures in adult flies, respectively. Furthermore, we found that h^{R95H} had a significant dominant-negative effect, rendering heterozygous flies susceptible to seizures and premature death. Using a recently developed dorsal vessel drug injection technique coupled with electrophysiological monitoring, we established that reduced GABAergic tone contributes to the seizure phenotypes in these knock-in lines. Together, these results provide biological bases for the various phenotypes resulting from multifunction of PNPO, specific molecular and/or genetic properties of each PNPO variant, and differential allele–diet interactions.

986F **Localization of Transgenes for *Drosophila* Models of Myotonic Dystrophy Type 1** Andrea Waltrip, Shyanne Michael, Delaney Baratka, Ginny R MorrissBiological Sciences, University of Mary Washington

Myotonic Dystrophy Type 1, DM1, is a multi-systemic disorder that results from expression of expanded CTG repeats in the *DMPK* gene in humans. *Drosophila melanogaster* is an established model organism for the study DM1, with three transgenic DM1 lines containing 60, 250, or 480 CTG repeats, expressed using the GAL4/UAS system. Expression of long-repeat transgenes ($(CTG)_{250}$ and $i(CTG)_{480}$) reproduces the phenotypes consistent with DM1, relative to control lines ($(CTG)_{60}$). The precise chromosomal location of insertion of the transgenes has not been reported. We are using both classical genetic and molecular approaches to localize CTG-repeat transgene insertion into the genome. To narrow down insertion location to a specific chromosome, we used GAL4 drivers on different chromosomes to drive expression of repeats and assessed phenotypic ratios of eye color, climbing capability, flight ability, and muscle degeneration, which is affected in DM1 flies. Results from the genetic analysis suggest that the $(CTG)_{250}$ and $i(CTG)_{480}$ transgene are likely localized to chromosome 2 and the $(CTG)_{60}$ transgene is likely localized to chromosome 3. We will confirm our chromosome localization using fluorescent in situ hybridization (FISH) of polytene chromosome preparations, using transgene-specific probes and comparing the hybridization location to regions that hybridize chromosome-specific probes. FISH will allow us to further narrow down the location of transgene insertion, allowing specific targeting of regions for sequencing to determine the exact insertion site. The information from this project is currently being used to set up mating schemes to assess how modulation of the pvr signaling pathway affects DM1-assoicated phenotypes. Knowing the location of the transgenes can allow for more practical mating schemes to study DM1 disease mechanisms, but also provides crucial information for understanding transgene expression, which may be influenced by nearby regulatory elements in the genome.

987F **Fly Fam161 is a Centriole and Connecting Cilium Protein Essential for Coordinated Behavior and Male Reproduction** Ankit Jaiswal[1], Andrew Boring[2], Tomer Avidor Reiss[31]Biological sciences, The University of Toledo, [2]The University of Toledo College of Medicine and Life Sciences, The University of Toledo Health Science Campus, [3]The University of Toledo

Fam161 is an ancient family of evolutionarily conserved proteins represented in mammals with two paralogs (FAM161A and FAM161B), studied only functionally in humans and mice. FAM161A is a centriole lumen protein thought to stabilize the centriole structure (Guennec et al., 2020). FAM161A is found also in photoreceptors connecting-cilium lumen and is essential for maintaining connecting-cilium stability and mammalian vision (Langmann et al., 2010) (Beryozkin et al., 2021). FAM161A is a component of the mammalian sperm typical and atypical centrioles, where it is found in unique luminal rod structures (Khanal et al., 2021). However, FAM161A essential role in the centriole is unknown.

Here, we use the fruit fly (*Drosophila melanogaster*) as a model to study the evolutionarily conserved role of Fam161, the ortholog of both mammalian paralogs. We use transgenic fly expressing Fam161GFP by Fam161 promoter or Fam161Neon by ubiquitin promoter, an antibody against N-terminus of Fam161, a likely null mutant with an early stop

codon *(fam161DN),* and a hypomorphic mutant lacking Fam161 C-terminus *(fam161DC)*.

We found that, like its mammalian ortholog, fly Fam161 is a centriole and connecting-cilium protein, suggesting this localization is evolutionary conserved. However, its localization in the connecting-cilium is cell-type specific. Consistent with Fam161 localization in sensory neurons, *fam161DC* has abnormal geotaxis, suggesting a reduced coordinated behavior. However, the behavioral phenotype is milder than mutation in proteins essential for centriole duplication and is simar to that of POC1B mutation, another centriole lumen protein.

F*am161DC* mutant males and females are fertile and 80% of fertilized eggs hatched larvae. However, only ~50% of the *fam161DC* males mate with control females within two hours compared to 80% mating success with wild-type males, suggesting that Fam161 is essential for efficient mating, probably due to its role in fly coordination. The F*am161DN* mutants (predicted to have only 59 of the 562 amino acids) were viable and exhibit similar general behavioral phenotypes to that of F*am161DC*. In the future, we want to study F*am161* role in sperm.

988F **A large genetic screen of human UAS-transgenes in *Drosophila* uncovers modifiers of Aβ42 and tau toxicity**
Vanlalrinchhani Varte, Jeremy W. Munkelwitz, Raymond Scott, Diego E Rincon-LimasNeurology, University of Florida

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by dementia and cognitive decline due to progressive cerebral cortical atrophy. Brains of AD patients are characterized by the accumulation of microscopic extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau. The deposition of Aβ42, which is one of the fragments of amyloid precursor protein (APP), has been known to play a role in initiating the events leading to the formation of amyloid and subsequently hyperphosphorylation of tau. However, animal models expressing either Aβ42 or tau individually do not mimic the complexity of the human condition. Indeed, recent evidence suggests that Aβ42 and pathological tau interact synergistically to modulate neurotoxicity in AD. To shed light on their concerted roles in AD pathogenesis and to discover pathways mediating Aβ42 and tau interactions, we generated transgenic flies co-expressing human Aβ42 fused to a signal peptide along with the longest wild-type tau isoform. Overexpression of Aβ42 or tau in *Drosophila* using the *UAS-Gal4* system causes mild to the moderate rough eye. In comparison, co-expression of Aβ42 with tau causes severe roughening and reduction of the eye size. The level of neuronal cell death in eye tissues was also significantly enhanced in flies co-expressing Aβ42 and tau. To identify pathways mediating Aβ42+tau interactions, we are currently using the Aβ42+tau eye phenotype as platform to screen 1,500 UAS lines expressing a variety of human genes. We have identified few enhancers and suppressors not previously known to be involved in AD pathogenesis, which will be helpful to uncover new molecular pathways and potential therapeutic targets. This work is supported by NIH grant R21AG069050 to DERL.

989F **From human to fly genetics: Identifying the genetic links between cardiovascular disease and insomnia**
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Cardiovascular disease (CVD) is a general term for a group of diseases that affect the heart or blood vessels, and is the leading cause of death worldwide. It is associated with insomnia where insomnia symptoms double the risk of incident CVD. However, the specific shared causal pathways remain poorly understood, making it difficult to identify new therapeutic targets that ameliorate insomnia-related CVD risks. Recently, two genome-wide association studies (GWAS) identified genetic loci significantly associated with insomnia symptoms, including one locus with 5 nearby genes that were previously reported as CVD-related genes in an independent GWAS. Therefore, we hypothesized that genetic predisposition to insomnia contributes to the development of CVD. To identify causal genes at this locus and understand the mechanisms linking CVD with insomnia, we used *Drosophila melanogaster* models, which are well-established model systems for sleep and cardiac studies. To assess the role of these genes on cardio-physiology,genetic knockdown (KD) was done using the cardiac-specific *Hand-Gal4* driver. 1-week-old *Drosophila* progeny were then used for semi-intact microscopic heart preparation followed by high-speed videography to assess cardiac physiological parameters. Similarly, to assess their role in sleep, KD of these genes was done using the neuron-specific *ELAV-Gal4* driver. Sleep and locomotor activity of 1-week-old flies was monitored using the *Drosophila* Activity Monitoring System. Our results show that neuronal and cardiac-specific RNAi knock-down of *Drosophila* orthologs of 4 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* suppressed hearts showed disruption in actin-containing myofibrillar organization. Suppression of *Lsn* increased Pericardin deposition, indicative of a fibrotic phenotype. Also, the cardiac suppression of *ATPSynC* and *Lsn* led to a significantly shortened lifespan. Additionally, neuronal-specific KD of 3 genes led to significantly decreased sleep compared to the RNAi control which was primarily due to a decrease in nighttime sleep, while KD of the fourth gene was lethal. This work will reveal novel genetic mechanisms linking CVD and insomnia.

990F **Neuronal mutant huntingtin causes synaptic loss and peripheral dysfunction in *Drosophila*** Jonathan R Roth, Ruan Carlos Macedo de Moraes, Brittney Xu, Malghalara Khan, Girish MelkaniPathology, University of Alabama at Birmingham

Huntington's disease (HD) is a devastating neurodegenerative disease characterized by movement and psychiatric dysfunction. HD is caused by a CAG expansion in exon 1 of the *HTT* gene leading to a polyglutamine (polyQ) repeat in the huntingtin protein, which aggregates in the brain. There is also evidence that peripheral dysfunction is common in HD as polyQ aggregates are found in the heart and muscle, and HD patients have a much higher chance of cardiac dysfunction than age-matched peers. Previously, we used *Drosophila* models to determine that polyQ aggregation in the heart leads to both structural and functional cardiac dysfunction and shortened lifespan. This dysfunction was ameliorated by promoting protein folding and reducing oxidative stress. Here, we further study the role of mutant huntingtin in the brain and how it affects peripheral function. We overexpressed normal (polyQ-25) or expanded mutant (polyQ-72) exon 1 of huntingtin in *Drosophila* neurons and found that mutant huntingtin causes age-dependent polyQ aggregation in the brain. This aggregation was associated with a loss of synapsin, indicating synaptic loss or dysfunction. To determine if this neuronal dysfunction in the brain could lead to peripheral dysfunction, we performed two measures of muscle performance in these flies: negative geotaxis to measure climbing muscle function, and flight index to measure flight muscle performance. We found that neuronal mutant huntingtin caused age-dependent climbing muscle performance dysfunction, and explored the role of autophagy in polyQ-induced dysfunction. These results demonstrate the importance of neuronal polyQ for both brain and peripheral function in models of HD and suggest that brain-periphery crosstalk could be important to the pathogenesis of HD.

991F **An RNA Polymerase subunit, Polr3c alters TDP-43^{M337V} toxicity in a fly model of ALS** Deepak Chhangani[1], Rogina Rezk[2], Akhil Patel[2], Aryan Shah[2], Swapnil Pandey[1], Lorena de Mena[2,2], Pedro Fernandez-Funez[3], Diego Rincon-Limas[11]Neurology, McKnight Brain Institute, University of Florida, [2]Neurology, University of Florida, [3]Biomedical Sciences, University of Minnesota

Tar DNA binding Protein-43 (TDP-43) is a major DNA/RNA binding protein involved in multiple cellular processes including transcriptional regulation, mRNA splicing and stress granules formation. Mutations in TDP-43, such as TDP-43^{M337V}, cause Amyotrophic Lateral Sclerosis (ALS). Abnormal accumulation and phosphorylation of TDP-43 is also associated with Frontotemporal Dementia (FTD) and Alzheimer's disease (AD). Despite its contributions to several devastating diseases, the toxic properties of TDP-43 are less understood, and hence, lesser is known about modifiers of its toxic effects. Here, we report the first genetic screen of over six thousand next generation RNAi lines in a Drosophila model expressing human TDP-43^{M337V}. We found ~200 genetic modifiers of TDP-43 toxicity using a degenerative fly eye phenotype as screening platform. We discovered a large number of genes encoding various transcription factor and RNA polymerase subunits, as robust modifiers of the proteinopathy in Drosophila eye. In particular, we found that knockdown of Polr3c suppresses TDP-43^{M337V} toxicity in eye and reduces phospho-TDP-43 staining in adult brain. Polr3c knockdown also significantly improves lifespan of flies with panneuronal expression of TDP-43^{M337V}. We anticipate that modifying or altering PolR3c can suppress the toxic effects caused by pathological TDP-43 mammalian models and may lead to the development of potential therapeutic approaches against TDP-43 proteinopathies. This work was supported by NIH grant R01059871.

992F **The cytoskeletal gene *shortstop* regulates Tau-induced neurodegeneration in *Drosophila* model of Alzheimer's Disease** Tom V Lee[1,2], Katherine Allison[1], Catherine Chen[3], Pritha Bagchi[4], Yarong Li[5], Ismael Al-Ramahi[1,2], Juan Botas[1,2], Nicholas Seyfried[4], Joshua M Shulman[1,21]Baylor College of Medicine, [2]Jan and Dan Duncan Neurologic Research Institute, [3]Rice University, [4]Emory University School of Medicine, [5]Neurology, Baylor College of Medicine

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of dementia. The genetic causes for the majority of AD cases and the underlying biology of pathogenesis remain unknown. Recent large scale proteomics studies on AD patient brains have identified multiple novel protein networks and targets that are

highly correlated with AD diagnosis and neuropathology. The most significantly AD-correlated protein network identified in this analysis contains the cytoskeletal protein Plectin (PLEC) as a protein network hub. PLEC functions to crosslink different components of the cytoskeleton and plays a role in axonal transport of synaptic proteins. We hypothesize that aberrant PLEC function increases AD susceptibility by disrupting cytoskeletal homeostasis and synaptic function. To validate Plectin as potential AD target of analysis, we performed functional analyses in *Drosophila* and human neuronal cell culture. The *Drosophila* homolog of Plectin, *shortstop*(*shot)* is ubiquitously expressed within the adult brain with strong enrichment in neuronal rich tissues. Using *Drosophila* models of human tauopathy, *shot* regulates age dependent Tau-induced neurotoxicity and locomotor dysfunction in a dosage sensitive manner. Plus, altering levels *shot* regulates Tau-phosphorylation, a marker for dysfunction, in both adult *Drosophila* brains and human neurons. In the absence of Tau overexpression, *shot* functions in light-induced neuronal transmission confirming its role in adult neurons. Taken together, we functionally validate *shot* as a regulator of Tau-induced neurodegeneration and modulator of Tau dynamics and neuronal homeostasis. Continued mechanistic analysis in both *Drosophila* and human neuronal cultures will determine *shot*'s role in AD neuropathogenesis and elucidate the underlying role of the cytoskeleton in Tau-induced neurodegeneration. To that end, we generated transgenic flies expressing an endogenous GFP-tagged version of Shot (*shot-GFP)* and performed immunoprecipitation-mass spectrometry (IP-MS) analysis to identify Shot protein interactors. Several cytoskeletal and synaptic proteins were identified and are currently being tested for their role in Tau-induced neurodegeneration and synaptic dysfunction.

993F **An *in vivo* screen identifies small molecule modulators of the endoplasmic reticulum stress response**
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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.

994F **Rare *de novo* missense variants in *DOT1L* are associated with developmental delay and facial dysmorphisms, and alter *grappa* activity in *Drosophila*** Zelha Nil[1,2], Ashish Deshwar[3,4], Scott Barrish[1], Yan Huang[1,2], Burak Tepe[1,2], Polona Le Quesne Stabej[5], Ian Hayes[6], Chad Haldeman-Englert[7], Carolyn Wilson[7], Trine Prescott[8], Kristian Tveten[8], Arve Vøllo[9], Devon Haynes[10], Patricia G Wheeler[10], Alexandra Afenjar[11], Mignot Cyril[12,13], Renaldo Florence[14], Boris Keren[15], Marwan Shinawi[16], Dustin Baldridge[16], James J Dowling[4,17], Daniel Wegner[16], Oguz Kanca[1,2], Michael F Wangler[1,2], Shinya Yamamoto[1], Gregory Costain[18], Hugo Bellen[11]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, [3]Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, [4]Program for Genetics & Genome Biology, The Hospital for Sick Children, [5]University of Auckland, [6]Genetic Health Service New Zealand, [7]Fullerton Genetics Center, [8]Telemark Hospital Trust, [9]Hospital of Østfold, [10]Arnold Palmer Hospital for Children, [11]Hôpital Trousseau, [12]Département de Génétique, Hôpital Trousseau, [13]13Centre de Référence Déficiences Intellectuelles de Causes Rares, [14]Service de Neurpoédiatrie, Hôpital Trousseau, [15]Hôpital de la Pitié-Salpêtrière, [16]Washington University School of Medicine, [17]Division of Neurology, The Hospital for Sick Children, [18]The Hospital for Sick Children

Misregulation of histone lysine methylation is associated with several human cancers and other disorders. *DOT1L* is an evolutionarily conserved gene encoding a lysine methyltransferase that methylates histone 3 lysine-79 (H3K79), and it has not been implicated in human genetic disorder previously. In this study, we identified seven unrelated individuals with *de novo* heterozygous missense variants in *DOT1L* through the Undiagnosed Disease Network (UDN) and GeneMatcher. All probands had some degree of global developmental delay/intellectual disability, and most had one or more congenital anomalies. We investigated the pathogenicity of the *DOT1L* variants using different alleles of its fly ortholog, *grappa* (*gpp*), and transgenic human *DOT1L* expressing fly lines. We find that complete loss or ubiquitous knockdown (<50%) of *gpp* causes a drastic decrease in H3K79 methylation levels, severe developmental delay, and lethality at larval stages. We also find *gpp* is broadly expressed in flies and primarily expressed in neurons in the central nervous system. Neuronal knockdown of *gpp* is lethal while glial knockdown does not cause any observable phenotype. Notably, wing specific overexpression of the human *DOT1L* variants causes blistering and necrosis in the wings similar to wing specific knockdown of *gpp*. Our data identify deleterious effects of patient variants on *DOT1L* function, and suggest that these variants are indeed pathogenic. Hence, *de novo* heterozygous variants in *DOT1L* are likely to be associated with a neurodevelopmental disorder.

995F **Repeated TBI leads to less severe acute outcomes, but worse long-term outcomes, than a single, severe TBI** Kamden T Kuklinski, Daniel Tulchinskiy, Rebecca DelventhalBiology, Lake Forest College

Traumatic brain injury (TBI) is a major cause of hospitalization, long-term disability, and death. However, it is challenging to discern the effect of different temporal patterns of injury because the timing, number, and severity of head injuries is so variable in a heterogenous human population. To address this, we used *Drosophila melanogaster* as a model organism to compare the outcomes of TBI administered over multiple days to the same TBI administered on a single day. We examined outcomes such as acute mortality, lifespan, locomotor performance, and expression of immune genes following the injury. Flies in the single-day TBI condition displayed higher acute mortality but longer lifespan than flies who received a multi-day TBI. The trend of worse long-term outcomes from an injury given over multiple days was also observed in locomotor performance. Flies given a TBI over multiple days climbed significantly worse than single-day TBI flies 2 weeks after TBI, but there was no difference between the two groups 48 hours post-TBI. To test the hypothesis that differences in the immune response to the two injury patterns may mediate the difference in short- and long-term outcomes, we measured expression of antimicrobial peptides (AMPs) at multiple timepoints post-TBI. We also examined the acute mortality of NF-κB pathway mutants in each injury condition to ascertain if the immune response plays a role in how different TBI patterns affect levels of acute death. A better understanding of the mechanisms underlying the differential outcomes of repeated TBI may enable development of treatments tailored to this pattern of injury.

996F ***nckx30c*, a *Drosophila* K^+-dependent Na^+/Ca^{2+} exchanger, is implicated in temperature-sensitive paralysis and age-dependent neurodegeneration** Al Nahian Khan Majlish[1], Shu Hui Lye[1], Emilia Cytron[1], Harris Bolus[1], Yeo Rang Lee[1], Kathleen Marotto[2], Atulya Iyengar[1], Stanislava Chtarbanova[11]Biological Sciences, The University of Alabama, [2]The University of Alabama

Clinical and research groups are becoming increasingly interested in the relationship between epileptic seizures and neurodegenerative disorders such as Alzheimer's Disease (AD). However, the mechanisms underlying this relationship are not fully understood. Ion channels that regulate intracellular calcium (Ca^{2+}) signaling are important in maintaining homeostatic ion balance in the nervous system. Disruption of ion channel activity can lead to neurological manifestations such as seizures. Moreover, imbalance in Ca^{2+} ion concentration is also linked to AD pathogenesis. *Drosophila* is a well-established model organism for successfully studying different human pathologies including neurodegenerative diseases and seizures. Prior studies have shown that temperature sensitive (TS) paralytic mutants, which exhibit a behavior equivalent of vertebrate epileptiform behavior, are enriched for neurodegeneration. To discover novel genes associated with both seizures and neurodegeneration, we performed an unbiased genetic screen on a collection of ENU-mutagenized *Drosophila* using the TS paralytic behavior at 38°C and subsequent histological analysis of brain tissue from isolated TS paralytic mutants. Candidate line 426 displayed TS paralysis as well as progressive neurodegeneration. A point mutation leading to a Threonine to Proline change in the ion binding domain of the K^+-dependent Na^+/Ca^{2+} exchanger *nckx30c* was mapped in exon 2 of the *nckx30c* gene. Examination of 426 and additional *nckx30c* alleles showed that in comparison to *wild type*, mutants had a shorter lifespan, decreased climbing ability, early-onset of neurodegeneration and changes in larval neuromuscular junction (NMJ) morphology, indicating potential synaptic dysfunction. Furthermore, knockdown of *nckx30c* specifically in neurons recapitulates the TS paralytic phenotype, whereas knockdown in both neurons and glia resulted in early onset climbing defects. The fly *nckx30c* gene is orthologous to mammalian Solute Carrier Family 24 (*SLC24*), whose function in the brain is poorly understood. Our research may help to shed more light on

SLC24 function while also establishing a link between seizures and neurodegeneration.

997F **Investigating pathogenic human CDK19 variant functions using Drosophila as a model organism** Zhe Liao[1], Hyunglok Chung[2], Claire RY Shih[1], Katherine Sew[1], Hugo J Bellen[2], Esther M Verheyen[11]Molecular Biology and Biochemistry, Simon Fraser University, [2]Molecular and Human Genetics, Baylor College of Medicine

Cyclin-dependent kinase 8 is a threonine/serine kinase that functions in a complex with its partners Cyclin C, Mediator 12 and Mediator 13. It is well-characterized in its mediator function as gene regulator; however, potential mediator-independent functions of Cdk8 have not yet been fully investigated. We found that expression of Cdk8 can modulate mitochondrial morphology under physiological condition. In muscles, depletion of *Cdk8* leads to an elongated mitochondrial phenotype, whereas expression of Cdk8 leads to a fragmented phenotype. A kinase dead version of Cdk8 further enhances the elongated phenotype, which suggests the change in mitochondrial morphology is likely to be a kinase-dependent event. In addition, when we examined the localization of Cdk8 using S2 cells, we found that Cdk8 is not solely localized in nuclei, which further suggests that Cdk8 may have a novel cytoplasmic function. Interestingly, in our collaboration with Dr. Bellen's lab on their project of CDK19, the human ortholog of fly Cdk8, they also found that CDK19 may have a novel cytoplasmic function. Two de novo mutations in *CDK19* are implicated in patient clinical features including epilepsy and neurodevelopmental delays. Furthermore, preliminary data suggests that cytoplasmically targeted CDK19 ($CDK19^{\Delta NLS}$) has similar abilities as the wildtype CDK19 ($CDK19^{WT}$) in rescuing neuronal defects caused by depletion of Cdk8, whereas two identified de novo mutations further enhance the defective phenotypes. To further validate these findings, we examined mitochondrial morphology and found fragmented mitochondria caused by $CDK19^{WT}$ and $CDK19^{\Delta NLS}$, whereas elongated mitochondria were found in fly cells expressing the patient variants. Excitingly, we observed the same elongated mitochondrial phenotype in patient-derived fibroblasts, which is consistent with our findings in flies. Besides investigating the novel cytoplasmic function of Cdk8/CDK19, we performed a drug test using N-acetylcysteine amide (NACA), which is an antioxidant drug that has previously proven to be protective against mitochondrial and neuronal defects. We tested if treatment with NACA can revert any defective phenotypes caused by Cdk8 dysfunction, and with the ultimate goal of providing potential therapeutic insights to patients with *CDK19* mutations. We will present our data on climbing, longevity, and mitochondrial morphology with the effects of NACA.

998F **Effects of Long-Term Nicotine Exposure on Adult *Drosophila melanogaster*** Blake A Tellinghusen[1], Luke A Bass[2], Norma A Velazquez Ulloa[31]Biology, Lewis & Clark College, [2]BCMB, Lewis & Clark College, [3]Biology, Lewis and Clark College

Despite the immense amount of research warning of the negative effects of its use, the tobacco epidemic continues, killing more than 8 million from direct use and 1.2 million from secondhand smoke exposure each year. Certain at risk-populations are especially vulnerable, including teens and children exposed during pregnancy. Nicotine, the highly addictive active ingredient in tobacco and e-cigarettes, is a plant-derived alkaloid that with continued use results in increased tolerance and reliance on the compound. While the receptors for nicotine have been established, there is still much to be determined regarding the genetic, cellular, and molecular mechanisms that mediate nicotine addiction. Previous studies have established *Drosophila melanogaster* as a viable model system to identify genes and novel mechanisms that control drug abuse. Our lab has shown that developmental nicotine exposure in *D. melanogaster* leads to a decreased survival rate and decreased sensitivity to nicotine and ethanol in adulthood. We are now characterizing the effects of long-term nicotine exposure in the adult stage. We performed a nicotine survival assay on adult male and female flies of the strain *w1118*, exposing them to nicotine-laced food with concentrations ranging from 0.0-1.3mg/mL, and found that increased nicotine concentrations lead to a higher mortality rate over long-term exposure. After a five-day nicotine exposure period (0.9mg/mL food), a negative-geotaxis assay and an olfactory choice assay were run on a separate group of male and female flies. We found that long-term adult nicotine exposure resulted in a slight increase in preference for grape odor in comparison to water odor for both sexes. In comparison we observed an increase in negative-geotaxis behavior in females only, possibly highlighting sex differences in long-term nicotine behavioral response. Next, we plan to run a nicotine CAFE assay to test for nicotine consumption escalation in adult flies, and to dissect and immunostain fly brains post-nicotine exposure to examine possible changes in dopaminergic neuron populations. Long-term nicotine exposure has been shown to elicit specific effects on *D. melanogaster* survivability and behavior, and with the additional characterization of these effects, we aim to further establish *Drosophila melanogaster* as a model organism for the identification of conserved mechanisms underlying nicotine's effects.

999F **Functional analysis of rare genetic variants in *SATB2* using *Drosophila melanogaster*** Hirokazu Hashimoto[1,2], Samantha L Deal[2,3], Oguz Kanca[1,2], Kenji Yokoi[4], Shinya Yamamoto[1,2,31]Molecular & Human Genetics, Baylor College of

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SATB2-associated syndrome (SAS, a.k.a. Glass syndrome: OMIM #612313) is a rare genetic disorder with over 550 known cases worldwide. This syndrome is characterized by developmental delay, intellectual disability with a severe speech impediment, autistic tendencies, psychiatric disturbances, and craniofacial dysmorphisms with variable expressivity and penetrance. Most cases are caused by *de novo* variants in *SATB2*. 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 a binary expression (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 (p.R239*and p.R459*) and three missense variants (p.R389C, p.E436V, p.G515S) previously reported in SAS patients. Based on *in vitro* studies, p.R239* was proposed to produce a truncated protein that acts in a dominant negative manner, whereas p.R459* has not been functionally studied. p.R389C and p.G515S are located in the first and second DNA-binding CUT domains of SATB2, respectively, and each has a different effect on the mobility of SATB2 within the nucleus when assessed in cellular models. Finally, p.E436V is a recently identified variant found in a unique SAS patient showing neurological symptoms without major dysmorphology. 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, p.R239* and p.R459* behaved as strong loss-of-function (LOF) alleles *in vivo*, rather than as dominant negative alleles. Second, p.R389C behaved as a milder LOF allele compared to the nonsense variants. Last, p.E436V and p.G515S behaved as gain-of-function (GOF) alleles.

In conclusion, some *SATB2* variants found in SAS patients behave as LOF alleles whereas others behave 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).

1000F **Rare *ZDHHC15* variants are associated with a human neurological disease based on fly studies** Mei-Chu Huang[1,2], Oguz Kanca[1,2], Matt Velinder[3], Lorenzo Botto[4], Michael Kruer[5,6], Hugo J. Bellen[1,2], Michael F. Wangler[1,2], Shinya Yamamoto[1,21]Department of Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Department of Human Genetics, University of Utah School of Medicine, [4]Division of Medical Genetics, Department of Pediatrics, University of Utah School of Medicine, [5]Pediatric Movement Disorders Program, Division of Pediatric Neurology, Barrow Neurological Institute, Phoenix Children's Hospital, [6]Departments of Child Health, Neurology, and Cellular & Molecular Medicine, and Program in Genetics University of Arizona College of Medicine

ZDHHC15 (*zinc finger DHHC-type palmitoyltransferase 15*) is one of 24 genes in the human genome that encodes a DHHC (aspartate-histidine-histidine-cysteine) motif-containing enzyme implicated in protein prenylation. This gene is located on the X chromosome and earlier human genetic studies suggested its involvement in intellectual disability. More recently, a male patient with multiple neurological symptoms including hypotonic cerebral palsy, focal-onset epilepsy, cortical visual impairment, intellectual disability, autism spectrum disorder, anxiety, and aggressive behaviors was found to carry a maternally inherited rare missense variant (p.H158R) in this gene. Through the Undiagnosed Diseases Network (UDN), we identified a female patient with multiple neurological symptoms including global developmental delay, febrile seizures, and absence epilepsy who carries a different *de novo* missense variant (p.P224L). While the missense variant identified in the male patient was shown to function as a strong loss-of-function (LOF) allele through studies performed in yeast, the functional consequence of the female patient's variant is unknown. Furthermore, while studies in flies, zebrafish, and mice suggest this gene is necessary for the proper development and/or function of the nervous system, the precise molecular function of ZDHHC15 *in vivo* is ill-defined.

To determine whether flies can be used as a tool to study the function of disease-associated ZDHHC15 variants, we generated transgenic *Drosophila melanogaster* strains that express reference or variant human ZDHHC15 based on the UAS/GAL4 system. We found that ubiquitous overexpression of reference ZDHHC15 causes lethality, and tissue-specific overexpression causes a variety of morphological defects. The p.H158R variant ZDHHC15 lacks this activity, consistent with this variant being a LOF allele. Interestingly, ZDHHC15 with the p.P224L variant causes stronger phenotypes when overexpressed compared to the reference protein, suggesting that this is likely to be a gain-of-function or dominant

negative allele. Currently, we are characterizing the expression pattern and the function of *CG1407*, the fly *ZDHHC15* homolog, using versatile *T2A-GAL4* lines to probe into the neuronal function and explore how the variants cause neurodevelopmental diseases in humans.

1001S **_Drosophila_ modeling reveals a conserved role for *ALG10/ALG10B* and the *N*-glycosylation pathway in the sleep-epilepsy axis.** Torrey R Mandigo[1,2], Shubhroz Gill[2], Brittany Leger[3], Bo Yang[4], Chanat Aonbangkhen[4], Suraj Math[1], Farah Abou Daya[5], Girish Melkani[5], Christina Woo[4], Stuart Schreiber[2], Richa Saxena[1], James Walker[11]Center for Genomic Medicine, Massachusetts General Hospital, [2]Broad Institute, [3]University of California: San Diego, [4]Harvard, [5]University of Alabama at Birmingham

Congenital disorders of glycosylation (CDG) comprise a class of inborn errors of metabolism resulting from pathogenic variants in genes coding for enzymes involved in the asparagine-linked glycosylation of proteins. Unexpectedly to date, no CDG has been described for *ALG10*, encoding the alpha-1,2-glucosyltransferase catalyzing the final step of lipid-linked oligosaccharide biosynthesis. Genome-wide association studies (GWAS) of human traits in the UK Biobank revealed significant SNP associations with short sleep duration, reduced napping frequency, later sleep timing and evening diurnal preference as well as cardiac traits at a genomic locus containing a pair of paralogous enzymes *ALG10* and *ALG10B*. Modeling Alg10 loss in *Drosophila,* we identify an essential role for the *N*-glycosylation pathway in maintaining appropriate neuronal firing activity, healthy sleep, preventing seizures and cardiovascular homeostasis. We present a previously reported human subject homozygous for a frameshift variant in *ALG10* arising from a consanguineous marriage, with epilepsy, brain atrophy and based on our *Drosophila* modeling, we predicted an additional mutation in *ALG10B* may be present. Sanger sequencing revealed a missense (p,(Leu253Trp)) variant in *ALG10B*. Furthermore, we predicted this patient may also have an undiagnosed sleep defect based on our findings in the human GWAS and fly modeling. Patient interviews and clinical sleep evaluations the patient was found to have 57% sleep inefficiency. Modeling of the patient variants in *Drosophila* demonstrated her *ALG10* frameshift was a strong loss of function while the *ALG10B*^{L253W} is likely a hypomorphic mutation. Quantitative glycoproteomic analysis in our *Drosophila* model identifies potential key molecular targets for neurological symptoms of CDGs, including an *N*-glycosylated carboxypeptidase which upon its knockdown recapitulates the *Alg10* knockdown phenotypes. An ongoing CRISPR-based structure-function analysis is being conducted to molecularly dissect important domains of the Alg10 enzyme.

1002S **Functional tests of variants of uncertain significance for genetic enhancement of muscle laminopathies** Nathaniel P Mohar[1,2], Sydney G Walker[1], Christopher J Langland[1], Benjamin W Darbro[2,3], Lori L Wallrath[1,21]Biochemistry and Molecular Biology, University of Iowa Carver College of Medicine, [2]Interdisciplinary Graduate Program in Genetics, University of Iowa, [3]Pediatrics, University of Iowa Carver College of Medicine

Mutations in the human *LMNA* gene encoding A-type lamins cause a collection of diseases known as laminopathies. These diseases affect many tissues including striated (skeletal and cardiac) muscle. A hallmark of these striated muscle laminopathies is their phenotypic variability. Individuals with the same *LMNA* mutation can have clinically distinct diseases. In addition, individuals with the same clinical diagnosis typically have dramatic variation in the severity of their muscle disease. This variability has long been attributed to genetic modifiers, but few specific modifiers have been identified to date. To identify genetic modifiers, we studied a family containing four siblings that possess the same *LMNA* mutation. Two of the siblings have severe Emery-Dreifuss muscular dystrophy, and two have very mild muscle defects. We have modeled this family's *LMNA* mutation in the Drosophila ortholog *LamC*. Muscle-specific expression of mutant *LamC* causes muscle defects similar to those observed in the human disease but without phenotypic variability among individual flies, further suggesting the genetic background is involved in the variability in humans. In an effort to identify genetic background differences that explain the variable phenotypes, whole genome sequencing was performed on the four siblings. Bioinformatic analysis identified a variant of uncertain significance (VUS) in *SMAD7* that co-segregates with severe muscle disease. *SMAD7* encodes a repressor of the TGFb/SMAD signaling pathway. Abnormal activation of this pathway is deleterious for differentiated muscle. CRISPR mutagenesis was used to make the identified variant in Drosophila *Dad*, the Drosophila ortholog of *SMAD7*. A combined model allowing for expression of the *LamC* mutation in tandem with the *Dad* variant has also been generated. Genetic tests are currently underway to determine if the *Dad* allele enhances the severity of the muscle defects caused by mutant lamin alone. The broad impact of *SMAD7* sequence variation was investigated through targeted sequencing of *SMAD7* in a cohort of 45 individuals with *LMNA*-associated muscular dystrophy. Additional VUS predicted to be deleterious were identified in moderate to severely affected individuals. Taken together, these studies strongly suggest variants in *SMAD7* enhance *LMNA*-associated muscle disease.

1003S **Biallelic variants in *INTS11* are associated with a novel complex neurological disorder** Burak Tepe[1,2],

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The integrator complex is a multi-subunit protein complex that is involved in processing of nascent RNAs transcribed by RNA polymerase II (RNAPII), including small nuclear RNAs, enhancer RNAs, telomeric RNAs, viral RNAs, and protein encoding mRNAs. *Integrator subunit 11 (INTS11)* is the catalytic subunit that cleaves nascent RNAs. Here, we describe 14 individuals from 10 unrelated families with biallelic variants in *INTS11*, who present with global developmental delay, language delay, intellectual disability, impaired motor development and pontocerebellar atrophy. We found that the fly ortholog of *INTS11*, *dIntS11*, is expressed in the central nervous systems in subset of neurons and most glia, at larval and adult stages. Loss of dIntS11 is lethal but can be fully rescued by a wildtype fly cDNA. Neuronal knockdown of dIntS11 does not cause any observable phenotype whereas 50% of the flies with glial knockdown fail to eclose, and the escapers mostly die 1 day post eclosion. We modeled the effect of seven variants on gene function by introducing the variants to the corresponding amino acids in dIntS11. Two of the variants tested (p.Arg17Leu and p.His414Tyr) failed to rescue lethality of null mutants indicating that they are strong LoF variants. Five of them (p.Gly55Ser, p.Leu138Phe, p.Lys396Glu, p.Val517Met, and p.Ile553Glu) rescued lethality but exhibit a short lifespan. When these variants are expressed at lower levels using GAL80ts, the flies displayed bang sensitivity and impaired locomotor activity indicating that they are partial LoF variants. In summary, we observed that the gene is expressed in most glia and that it is essential in glia. Moreover, our data strongly argue that biallelic variants in INTS11 result in a novel complex neurological disorder.

1004S **De Novo Variants in MRTFB have gain of function activity in Drosophila and are associated with a novel neurodevelopmental phenotype with dysmorphic features.** Jonathan Andrews[1,2], Jung-Wan Mok[1,2], Oguz Kanca[1,2], Sharayu Jangam[1,2], Cynthia Tifft[3], Ellen F Macnamara[4], Bianca Russell[5], Lee-Kai Wang[6], Stanley Nelson[5], Hugo J Bellen[1,2,7], Shinya Yamamoto[1,2,7], May Malicdan[3], Michael F Wangler[1,21]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, [3]Pediatric Undiagnosed Diseases Program, NHGRI, [4]Pediatric Undiagnosed Diseases Program, NIH, [5]Department of Pediatrics, University of California, Los Angeles, [6]Institute for Precision Health, University of California Los Angeles, [7]Department of Neuroscience, Baylor College of Medicine

Myocardin-related transcription factor B (MRTFB) is a member of a family of genes which serve to potentiate serum response factor (*SRF*)-dependent transcription and is highly conserved in both vertebrate and invertebrate model organisms. MRTFB is not currently associated with a human disease but has been shown to be highly expressed in all human tissues save the lung. Here we report the successful generation of a *Drosophila* model of MRTFB-related disorders, modeling a pair of probands with a *de novo* variants (p.R104G and p.A91P) in the second RPEL domain of *MRTFB*. Using the GAL4/UAS system to drive the pathogenic variants within wing tissues via a *Nubbin-Gal4* driver was sufficient to induce significant morphological changes in the fly wing, including truncations of wing veins, expansion of intervein space, loss of crossveins, and blistering. Conversely, expression of a reference human *MRTFB* cDNA produced only minor changes in the posterior crossvein, and expression of *Drosophila Mrtf* produced a similar change in crossvein length as was observed with the human reference. To identify if this change in wing morphology was due to a lack of

RPEL domain functionality, a *UAS-MRTFB$^{\Delta RP2}$* line was established which lacked the entire 2nd RPEL domain. Expression of this line within the flies wings resulted in damage which was indistinguishable from the changes in morphology caused by the expression of either the p.R104G or p.A91P variants. In *Drosophila*, the *SRF* ortholog, *blistered (bs),* is known to suppress wing vein formation and promote the development of intervein cells. Exogenous co-expression of *bs* and *Mrtf* has been previously shown to significantly alter wing morphology; therefore we expressed the human reference and variant cDNA lines concurrently with a UAS-*bs* line. We found that wing morphology was highly disrupted when *bs* and the reference human cDNA were co-expressed, while the co-expression of p.R104G or p.A91P variant cDNA and *bs* was lethal. As the interaction between *MRTFB* and *SRF* is dependent on actin binding within the RPEL domain, *MRTFB* variants could disturb actin binding. We identified a significant decrease in the ability of the p.R104G and p.A91P variants to bind to actin, suggesting that a lack of regulation may cause its effects on the fly wing. This lack of binding also had clear consequences for downstream targets of *mrtf*, including an increase in the level of *Actin5c* transcripts, which could be clearly visualized within ovary cells. Our results clearly demonstrate that the p.R104G and p.A91P variants disrupt actin binding and underlie a novel disorder.

1005S **A *Drosophila* model of PIGA deficiency, a rare X-linked intellectual developmental disorder, reveals that distinct PIGA deficiency phenotypes arise from independent cell types** Emily Coelho, Maddie Haller, Clement Y. ChowUniversity of Utah Human Genetics

Mutations in the *Phosphatidylinositol glycan class A* (*PIGA*) gene cause PIGA deficiency, an x-linked epilepsy and intellectual developmental disorder. PIGA deficiency is classified as a congenital disorder of glycosylation (CDG) and it shares many symptoms with other CDGs such as shortened lifespan, hypotonia, dysmorphic features, epileptic seizures and a number of congenital anomalies. Like other CDGs, PIGA deficiency is a rare disease with fewer than 100 patients reported. *PIGA* encodes an enzyme that catalyzes the first step in glycosylphosphatidylinositol (GPI) anchor biosynthesis by transferring a N-acetylglucosamine to phosphatidylinositol. GPI attaches the C-terminus of a protein to the cell surface. GPI-anchored proteins play a number of roles in cell signaling, migration, and immunity. It remains unclear how loss of PIGA function contributes to the phenotypes observed in patients. Because a number of the patient phenotypes include nervous system abnormalities, we used RNAi technology to knockdown *Drosophila PIGA* (*PIG-A*) expression in neurons and in glia. Neuron-specific loss of *PIGA* function results in reduced lifespan, neuromuscular defects and neurological abnormalities, including wing position defects and sleep disturbances. Because epilepsy is present in all PIGA deficiency patients, it is surprising that neuronal knockdown does not result in a seizure-like phenotype. Strikingly, glia-specific knockdown does result in a seizure-like phenotype as well as a wrinkled wing phenotype, but no movement disorder. To understand the molecular underpinnings of these cell type-specific phenotypes, RNAseq analysis was performed on heads from flies with neuronal or glia-specific knockdown of *PIGA* and controls. Transcriptome differences between neuron vs glial knockdown heads revealed potential mechanisms as to why seizures are observed in glial knockdowns and not in neuronal knockdowns. These models provide a path forward for precision medicine approaches in PIGA deficiency, including patient specific modeling and small molecule screens.

1006S **Detecting phenotypic differences of Alzheimer's progression using GAL4-UAS lines in Drosophila** Michaela Jemison[1], Samantha Scoma[2], Krishna Bhat[2], Alexis Nagengast[21]Biology, Widener University, [2]Biochemistry, Widener University

Alzheimer's Disease is a progressive neurodegenerative disease ranked as the sixth leading cause of death in the United States. Utilizing the GAL-UAS system, we are testing transgenic models of Alzheimer's Disease with the expression of the human APP-Aβ42 gene in the eye of *Drosophila melanogaster*. Resveratrol, a naturally occurring compound found in red wine, is known for its anti-aging properties and may be beneficial in the quest for discovering better treatment options for Alzheimer's Disease. Drugstore supplements of resveratrol are available but contain little active compound partially due to the high cost of extraction and purification. We have synthesized derivatives of resveratrol via a modified Wittig Reaction, with a special focus on cost effectiveness and the application of green chemistry principles. We have performed food consumption assays and verified *Drosophila* ingest a yeast mixture containing resveratrol or derivative. We have established that there are no adverse effects associated with consumption, and wildtype flies have a normal life span of approximately 70 days. Flies of the genotypic background GAL4-GMR/UAS-APP-Aβ42 were phenotypically observed for eye degeneration and flies of the genotypic background GAL4-Elav/UAS-APP-Aβ42 were studied via locomotion assays. We have observed eye degeneration in the GMR model and have found resveratrol treatments improve locomotion in Elav model flies. This work will be continued in order to find an optimal dosage of resveratrol in flies, and to test the efficacy of the derivatives in the prevention or slowing of Alzheimer's Disease symptoms in *Drosophila* models.

1007S ***EZH1-variant* related developmental delay case-study displays functional deficits in *Drosophila melanogaster*** Sharayu Jangam[1,2], Lauren C Briere[3], Kristy Jay[1], Melissa A Walker[4], Jonathan Andrews[1], Frances A High[5], Lance Rodan[6], Hugo Bellen[1,2], Shinya Yamamoto[1,2,7], David A Sweetser[3,5], Michael F Wangler[1,2,71]Dept. of Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Neurological Research Institute, Texas Children's Hospital, [3]Center for Genomic Medicine, Massachusetts General Hospital, [4]3Department of Neurology, Division of Neurogenetics, Child Neurology Massachusetts General Hospital, [5]Division of Medical Genetics & Metabolism, Massachusetts General Hospital for Children, [6]Department of Neurology, Boston Children's Hospital, [7]Genetics and Genomics program, Baylor College of Medicine

DNA histone modifiers play a very critical role during the embryonic development of an organism. *EZH1 (Enhancer of Zeste homologue, 1)*, along with it's partners - *EED, SUZ12*, and *RBAP46/48,* as component of a Polycomb Repressive Complex-2 (PRC2) group, promotes the maintenance of embryonic stem cell pluripotency by modifying Lysine27 at histones 3 (H3K27). *EZH1* in this complex, with its methyl transferase activity, suppresses downstream target genes by H3K27-trimethylation (H3K27me3). Genetic mutations in such DNA histone modifiers have been shown to disrupt key signaling pathways leading to developmental disorders but *EZH1* has not yet been linked to disease. Here we report a *de novo* variant in *EZH1-p.Ala678Gly*, resulting in a novel neurodevelopmental phenotype. The patient, who was evaluated at 2.5 years of age, has a severe global developmental delay, mixed tone, proximal muscle weakness, and intermittent exotropia. While there is no history of seizures, EEG was indicative of mild diffuse cerebral dysfunction. The patient also has mild short stature with preserved head circumference, small hands and feet, mild dysmorphisms, and hypopigmentation. This variant, p.A678G*,* is in a well-conserved motif, the SET domain, which is required for its methyltransferase activity. Human *EZH1* is homologous to fly *Enhancer of zeste E(z)* (DIOPT: 11/16), and the residue (A678 in humans and A691 in *Drosophila*) is also conserved. To study its functional aspect *in vivo*, we generated transgenic flies expressing wild-type *(E(z)^{WT})* and the variant *(E(z)^{A691G})* in *Drosophila.* When it is constitutively active under the tubulin promotor, the variant rescued the fly null lethality similar to wild-type suggesting the variant form encodes a functional protein. However, hyper H3K27me3 was seen in *E(z)^{A691G}* and not in *E(z)^{WT}.* Moreover, the *E(z)^{A691G}* flies were not entirely healthy, exhibiting bang sensitivity and shortened lifespan. In conclusion, a novel *EZH1 de novo* variant in a neurodevelopmental syndrome exhibits functional impact in *Drosophila* with potential gain of function effects.

1008S **HAP40 is a conserved central regulator of Huntingtin and a potential modulator of Huntington's disease pathogenesis** Shiyu Xu[1], Gang Li[1], Xin Ye[1], Dongsheng Chen[1], Zhihua Chen[1], Moretti Daniele[2], Sara Tambone[2], Alessandra Ceccacci[2], Licia Tomei[2], Lili Ye[1], Yue Yu[1,3], Amanda M Solbach[1,3], Stephen M Farmer[1,4], Erin Furr Stimming[5], George McAllister[6], Deanna M Marchionini[6], Sheng Zhang[1,7,81]Institute of Molecular Medicine, The University of Texas Health Science Center at Houston (UTHealth), [2]Department of Translational and Discovery Research, IRBM SpA, [3]Neuroscience Program, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS), [4]Program in Biochemistry and Cell Biology, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS), [5]Department of Neurology, HDSA Center of Excellence, The University of Texas Health Science Center at Houston (UTHealth), [6]CHDI Management/ CHDI Foundation, [7]Department of Neurobiology and Anatomy, The University of Texas Health Science Center at Houston (UTHealth), [8]Programs in Genetics and Epigenetics and Neuroscience, The University of Texas MD Anderson Cancer Center UTHealth Graduate School of Biomedical Sciences (MD Anderson UTHealth GSBS)

Perturbation of huntingtin (HTT)'s physiological function is one postulated pathogenic factor in Huntington's disease (HD). However, little is known how HTT is regulated *in vivo*. In a proteomic study, we isolated a novel ~40kDa protein as a strong binding partner of *Drosophila* HTT and demonstrated it was the functional ortholog of HAP40, an HTT associated protein shown recently to modulate HTT's conformation but with unclear physiological and pathologic roles. We showed that in both flies and human cells, HAP40 maintained conserved physical and functional interactions with HTT. Additionally, loss of HAP40 resulted in similar phenotypes as HTT knockout. More strikingly, HAP40 strongly affected HTT's stability, as depletion of HAP40 significantly reduced the levels of endogenous HTT protein while HAP40 overexpression markedly extended its half-life. Conversely, in the absence of HTT, the majority of HAP40 protein were degraded, likely through the proteasome. Further, the affinity between HTT and HAP40 was not significantly affected by polyglutamine expansion in HTT, and contrary to an early report, there were no abnormal accumulations of endogenous HAP40 protein in HD cells from mouse HD models or human patients. Lastly, when tested in *Drosophila* models of HD, HAP40 partially modulated the neurodegeneration induced by full-length mutant HTT while showed no apparent effect on the toxicity of mutant HTT exon 1 fragment. Together, our study uncovers a conserved mechanism governing the stability and *in vivo* functions of HTT and demonstrates that HAP40 is a central and positive regulator of endogenous

HTT. Further, our results support that mutant HTT is toxic regardless of the presence of its partner HAP40, and implicate HAP40 as a potential modulator of HD pathogenesis through its multiplex effect on HTT's function, stability and the potency of mutant HTT's toxicity.

1009S **Interactions between stv and p38Kb as a Charcot-Marie-Tooth disease model** Shira Archie[1], Alysia Vrailas-Mortimer[21]Illinois State University, [2]Oregon State University

Many age-related and degenerative diseases, such as Charcot-Marie-Tooth disease (CMT), are the result of irregular protein aggregations. One mechanism by which protein aggregation is regulated in mammalian systems is the BAG-3 Mediated Selective Autophagy pathway, known as the Chaperone-Assisted Selective Autophagy (CASA complex) in flies. We have recently found that the CMT protein lamin is a target of p38Kb and the CASA complex member, starvin (stv), for degradation during aging. CMT is a group of inherited disorders that causes axonal degeneration and demyelination of the peripheral motor and sensory neurons. Symptoms include progressive motor impairment, distal muscle weakness, sensory loss, reduced tendon reflexes, and foot deformities. We are currently performing studies to determine the exact binding site between p38Kb and stv and the mechanism in how they bind. This information is crucial to understand the system in which protein aggregation occurs in CMT, and potentially how to rescue the degenerative phenotypes associated with the incurable disease.

1010S **Cellular progression of the huntingtin protein throughout development and its impact on the health and physiology of Drosophila melanogaster** Tadros Hana, Kiel OrmerodBiology, Middle Tennessee State University

Huntington Disease (HD) is an inherited disease, affecting 1/10000-1/20000 people yearly, highlighted by a progressive breakdown of the cells in the brain, leading to progressive loss of motor control, cognitive decline, and ultimately death (Roos, R.A, 2010). HD currently has no cure and the exact mechanism behind its pathology remains unclear at best (Nupoulos, P.C. 2016). Unlike most neurodegenerative diseases, a gene associated with the pathogenicity has been identified, the Huntington gene, which encodes the Huntingtin protein (htt). The disease is attributable to an abnormal expansion in one part of the gene, the polyglutamate (PolyQ) tract, which leads to misfolding and aggregation of the protein within nerve cells (Arraste and Finkbeiner, 2011; Gatto et al, 2020). The severity of the disease and the age of onset have been shown to correlate with an expansion within a pivotal region of the Huntington gene, the PolyQ tract (Langbehn et al, 2010). Our lab models HD in Drosophila Melanogaster by examining the molecular and cellular progression of htt aggregation and the physiological and behavioral impacts. Using two transgenic fly lines, one with a normal number of glutamine residues (Q15) and one with a pathogenic number (Q138); both constructs include a red fluorescent tag to the end of the protein to visualize its cellular location (Weiss et al, 2012). Here we monitor the progression of cellular accumulation of htt-aggregates and determine if a correlation exists between the size/number of cellular aggregates and severity of physiological and morphological impacts. We monitored the progression of htt-aggregates throughout the development of the organism at each larval stage using live confocal and fluorescent imaging and demonstrate a development correlation with htt-aggregation. Additionally, we explored the effects of htt-aggregates on overall health by examining established morphological features (Ormerod et al., 2017). The results from this study deepen our understanding of the pathology of Huntington's Disease and establish a relationship between aggregates' size/number and their broader impact on the organism's health and physiological integrity.

1011S **Knockdown of *Tpr2* Rescues *Drosophila* Models of ALS** Jonah F. Boardman[1,2], Kristi A. Wharton[1,21]Department of Molecular Biology, Cell Biology, and Biochemistry, Brown University, [2]Carney Institute for Brain Science, Brown University

The integrity of cellular proteins is maintained by molecular chaperones such as heat shock proteins (HSPs) that regulate protein folding and degradation. Hsp70 and Hsp90 are two major families of HSPs that can coordinate in the folding of clients. Hsp40 is a family of co-chaperones which regulates chaperone activity. The well-studied Hsp70/Hsp90-organizing protein (STIP1) acts in the Hsp70/Hsp90 folding pathway by passing clients from Hsp70 to Hsp90. DnaJ heat shock protein family (Hsp40) member C7 (DNAJC7), or TPR2, contains both a J-domain required for interactions with Hsp70 and two tetratricopeptide domains which recognize Hsp90. It is believed to act in the opposite direction of STIP1 in the Hsp70/Hsp90 folding pathway by passing clients back to Hsp70 from Hsp90 (Brychzy et al., 2003). Recent studies have identified DNAJC7 truncation mutations in ALS patients, implicating a potential loss of function role of this Hsp40 family member in neurodegeneration and disease progression (Farhan et al., 2019). Interestingly, we found that increasing the levels of *Tpr2,* the *Drosophila* ortholog of DNAJC7*,* by overexpression, did not prevent ALS-associated degeneration. Instead, we demonstrated that increased expression of *Tpr2* can be detrimental in both the diseased and non-diseased

state. In contrast, we found that *Tpr2* knockdown suppresses the shortened lifespan of an adult onset *C9orf72-G_4C_2-* repeat expansion model of ALS as well as defects associated with three other *Drosophila* ALS models: hTDP43[M337V], hFUS[R521C], and dSod1[G85R] knock-in. Based on the model proposed by Brychzy et al., we hypothesize that the refolding process instigated by Tpr2 may be disadvantageous for irreparable proteins like those expressed in ALS. For these proteins, an increase in Tpr2 may result in continuous refolding attempts that serve no benefit to the cell. Instead, this process may deplete the supply of chaperone proteins needed for refolding of salvageable clients.

1012S **An inducible Drosophila model to identify genetic modifiers of insulin deficiency** Yuyan Chen, Xuan ZhuangUniversity of Arkansas

Diabetes and related metabolic disorders are complex diseases influenced by a variety of factors, interactions among genetic elements, and between genes and the environment. Even the same genetic defect could demonstrate substantial phenotypic variability in human populations with different genetic backgrounds. Therefore, it is crucial to understand how genetic background interacts with major disease-causing factors to produce differential susceptibility to disease. Here we develop a *Drosophila* model by incorporating a disease-causing genotype into a natural population with different genetic backgrounds. Genetically modified *Drosophila can* model diabetes by ablating insulin-like peptide-producing cells (IPC). Two transgenes, *UAS-reaper* and *Dilp2-GeneSwitchGAl4(GS)*, were introduced causing IPC apoptosis, which results in a reduction of insulin-like peptides and affects metabolic homeostasis. This controllable mutated genotype needs to be dominant and can be transferred, as a whole, to express in flies with different genetic backgrounds. To link the two transgenic mutations (*dilp2*-GS and UAS-*rpr*) onto a single chromosome, we generate a recombinant tested line (*dilp2*-GS, UAS-*rpr*/*CyO*) by a series of genetic crosses. PCR and sequencing results confirm the presence of two correct transgenes in the constructed recombinant tested line. The IPC-ablated flies demonstrate a substantially decreased expression of *Drosophila* insulin-like peptide2 (*dilp2*), and an increase in glucose and triglycerides levels, which mimic the diabetic phenotypes. Together, we have successfully developed a fly disease model with adult-specific insulin-like peptides knockdown using genetic cross and recombination techniques. Furthermore, we will introduce the IPC-ablated genotype to the *Drosophila* Genetic Reference Panel to study the genetic modifiers of insulin deficiency.

1013S **Linking disruptions in synaptic vesicle trafficking to neurodegeneration: implications for a noncanonical endocytic regulator, Tsp42Eg** Emily L Hendricks, Faith LieblBiological Sciences, Southern Illinois University Edwardsville

Dynamic readily releasable and reserve vesicle pools are maintained at synaptic terminals and are important for neurotransmitter release during basal and high frequency stimulation, respectively. The replenishment of these synaptic vesicle pools is highly dependent on endocytic processes and vesicle trafficking mechanisms. Thus, proper neurotransmission at the synapse relies on the coordinated cycling of vesicles through endocytic pathways. In cases of neurodegenerative diseases, like Alzheimer's disease, pathological protein aggregates disrupt endosomal trafficking and impair overall synaptic function. We have identified a late endosome-associated protein, CD63, that may functionally interact with amyloid beta (Aβ)—a pathological protein fragment implicated in Alzheimer's disease. To explore the synaptic function of CD63, we use the *Drosophila* neuromuscular junction (NMJ) as a model glutamatergic synapse. We find that the CD63 homolog, Tsp42Eg, is expressed transsynaptically and suppresses Aβ accumulation at the synapse. Furthermore, we find that Tsp42Eg negatively regulates endocytosis and promotes vesicle recycling to reserve and readily releasable vesicle pools. However, it is unclear if loss of *tsp42Eg* at the synapse directly disrupts these synaptic processes or if Aβ accumulation at *tsp42Eg* mutant synapses imparts the observed synaptic dysfunction. A potential molecular target of Aβ pathology is the reserve pool-associated protein, Synapsin. To discern the relationship between Tsp42Eg and Aβ, we assess synaptic Synapsin localization in *tsp42Eg* mutants and in animals where Aβ accumulation is genetically promoted through the overexpression of APP and BACE (APP BACE OE). In both *tsp42Eg* mutants and APP BACE OE animals, we observe an increase in synapse-localized Synapsin. These findings suggest that Aβ acts upstream of Tsp42Eg to affect Synapsin. These findings further suggest that Tsp42Eg's repression of endocytosis, rather than any regulatory actions on Synapsin, may prevent synaptic Aβ accumulation. Future work will use both genetic and pharmacologic inhibition of Synapsin activity and endocytosis in *tsp42Eg* mutants to further elucidate Tsp42Eg's role in synaptic Aβ regulation. More broadly, this work will further clarify mechanisms of vesicle trafficking disruption in Alzheimer's disease pathology.

1014S **A Malpighian tubule phenotype in *parkin* mutant *Drosophila melanogaster*** Samantha M. Chagolla, Krista Pearman, Geral B. CallMidwestern University

Parkinson's Disease (PD) is an age-related neurodegenerative disease that affects millions worldwide. Although the most noticeable symptoms are associated with dysregulation of motor function, non-motor function symptoms are also present and can be debilitating. A few of these less well-known, non-motor symptoms include constipation, loss of olfaction, and urinary issues. In fact, many PD patients have non-motor symptoms years before any visible motor dysfunction symptoms. Autosomal-recessive juvenile Parkinsonism is caused by genetic mutations in a variety of genes, one of which is *PRKN*. Mutation of the *parkin* (*park*) gene, the *Drosophila melanogaster* ortholog of the human *PRKN* gene, can cause many phenotypes that are similar to PD symptoms, including selective loss of dopaminergic neurons, impaired motor function, olfaction loss, decreased lifespan, and mitochondrial dysfunction. Here, we report a new phenotype in homozygous $park^{25}$ (a null allele) flies in the Malpighian tubule (MT). The MT is the excretory organ of the fly and thus considered analogous to the kidney. The MTs in combination with the hindgut are responsible for the filtration of the hemolymph and regulation of ionic and osmotic homeostasis. There are two anterior and two posterior blind-ended tubules that drain into the gut at the midgut/hindgut border. These simple tubules are very small, being essentially cellular monolayers and typically only 3mm in length. The MT is made up of three known cell types, principal cells, stellate cells and renal stem cells. Initial observation of the MTs in the homozygous $park^{25}$ fly reveals an enlarged organ, sometimes similar in size to the neighboring gut. This enlargement can also be observed in $park^{25}$/Df(3L)BSC553 trans-heterozygous flies that also lack the *parkin* gene. Additionally, the MTs in $park^{25}$ flies also have a number of opaque masses within the lumen that can sometimes be quite large. While liquid within the lumen of the MT in control flies can sometimes appear opaque, this opacity will disappear upon placing the MT in 50% glycerol for microscopic observation. However, the opaque masses in the $park^{25}$ flies do not disappear in glycerol, possibly indicating that they are solid structures that may be similar to kidney stones. Further characterization of these MT phenotypes will be performed and presented at the upcoming meeting.

1015S **Development of *Drosophila* model for retinal vasculopathy and cerebral leucoencephalopathy (RVCL)** Elena Gracheva, Abigail Matt, Joanna Chen, Matthew Fishman, Fei Wang, Chao ZhouWashington University in St Louis

Retinal vasculopathy with cerebral leukoencephalopathy (RVCL, also known as RVCL-S or HERNS) is a rare disease that affects 100% of people with mutations in a specific region of the *TREX1* gene. RVCL manifests mainly in middle age individuals; in most patients death occurs 5 to 10 years after onset. The underlying mechanisms of disease pathogenesis are still unknown, partly due to the lack of robust animal models. Using bioinformatics methods, we have identified *cg3165* gene as a *Drosophila* homolog of human *TREX1* gene. To investigate the significance of fly TREX1 (CG3165) in development and normal organism function, we performed the longevity study and have found that CG3165 knock-down causes higher mortality rates. Major RVCL symptoms are linked to a vascular system. *Drosophila* has a straightforward vascular system, consisting of an aorta and a heart tube located on the dorsal side of the insect within 200 µm from the tissue surface. Our lab has developed a non-invasive imaging technology, Optical Coherence Tomography, and successfully demonstrated morphological and functional changes of the *Drosophila* heart throughout all the developmental stages. We used *Act5C>TREX KD* flies to assess the function of the aging fly cardiovascular system. We set up the evaluation protocol that includes several parameters, such as: arrhythmia index, fractional shortening, and the heart rate. The results summary for 1 week old TREX KD flies have shown more consistent heart rhythm than WT control flies, but fractional shortening data suggested a trend to a contraction reduction. The heart rate is also reduced in TREX KD flies. Overall, *D. melanogaster* model organism provides an efficient and informative platform to study RVCL pathological features.

1016S **Kekkon5's interaction with dopaminergic signaling on inhibitory control** Bryan A Hernandez, Erick B Saldes, Paul Sabandal, Kyung-An HanBiological Sciences, The University of Texas at El Paso

Inhibitory control is a core executive function important for goal-directed behaviors. Dysfunctional inhibitory control is associated with numerous neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASD). ADHD and ASD involve altered dopamine signaling; however, the mechanism by which altered dopamine signaling leads to dysfunctional inhibitory control remains largely unknown. To address this gap in knowledge, we performed functional screens to identify novel genes interacting with the dopamine system for inhibitory control. We identified kekkon5 (kek5), a homolog of human LRFN1 gene, as one of the genes interacting with the dopamine transporter mutation for inhibitory control. Kek5 is a cell adhesion molecule and functions as a negative regulator of BMP signaling. The heterozygous kek5 (kek5/+) or fumin (fmn/+) mutant showed normal inhibitory control, however the double heterozygous kek5/+;fmn/+ mutant displayed dysfunctional inhibitory control. The progress including the neural site for the kek5 and dopamine interaction with as well as the mechanism underlying their interaction for inhibitory control will be presented. This study will provide insight into the mechanism by which cell

adhesion molecules interact with the dopamine system for inhibitory control.

1017S **Rare *de novo* missense variant in *IP6K3* is associated with developmental delay and microcephaly, and causes neurological phenotypes in *Drosophila*** Megan A. Cooper[1,2], Zelha Nil[1,2], Jonathan Andrews[1,2], Daryl A. Scott[1,3,4], Undiagnosed Diseases Network[5], Shinya Yamamoto[1,2,6,7], Michael F. Wangler[1,2], Oguz Kanca[1,2], Hugo J. Bellen[1,2,6,71]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [3]Texas Children's Hospital, [4]Molecular Physiology and Biophysics, Baylor College of Medicine, [5]Harvard Medical School, [6]Neuroscience, Baylor College of Medicine, [7]Development, Disease Models & Therapeutics Graduate Program, Baylor College of Medicine

Inositol pyrophosphates (PP-IPs) are high-energy signaling molecules, which are known to serve important roles in many biological processes such as energy metabolism and exocytosis in neurons. In mammals, the Inositol hexakisphosphate kinase family (IP6Ks) consists of *IP6K1-3* and generate PP-IPs from inositol phosphates (IPs). Recent studies have demonstrated the importance of *IP6K1* and PP-IPs in regulating synaptic vesicle exocytosis in mice. Additionally, mouse *IP6K*3 has been shown to regulate synapse formation of cerebellar Purkinje cells, leading to deficits in motor learning and coordination. Thus far, *IP6K3* has not been implicated in a human genetic disorder. In this study, we identified an individual with a *de novo* heterozygous missense variant in *IP6K3* through the Undiagnosed Disease Network (UDN). The proband had developmental delay, microcephaly, and congenital anomalies. We investigated the pathogenicity of the patient's variant using different alleles of its fly ortholog, *Ip6k* (CG10082), and transgenic human *IP6K2* and *IP6K3* expressing fly lines. *Ip6k*^{T2AGAL4} loss of function (LOF) allele over a molecularly defined deficiency (*Df*) covering *Ip6k* is semi-lethal (<50%), indicating that *Ip6k* is important for proper development of many animals. Surviving flies display increased bang sensitivity, reduced climbing ability and a reduction in lifespan. *Ip6k*^{T2AGAL4} / *Df* phenotypes can be rescued by expressing either UAS-IP6K2^{ref} or UAS-IP6K3^{ref} human cDNAs indicating that the molecular function of IP6Ks is evolutionarily conserved. Conversely, expressing the variant in the *Ip6k*^{T2AGAL4} / *Df* flies does not rescue the LOF phenotypes and indeed exacerbates them. Expressing UAS-IP6K3^{var} together with either IP6K2^{ref} or IP6K3^{ref} does not impair the rescue of *Ip6k*^{T2AGAL4} / *Df* phenotypes, suggesting that the variant does not act as a dominant negative. Together, our data suggest that the patient variant is a gain-of-function allele.

1018S **A Genetic screen to identify therapeutic targets for a neurodevelopmental syndrome** Udai Pandey[1], Tyler Fortuna[1], Sukhleen Kour[1], Anuradha Venkatakrishnan Chimata[2], Anixa Muiños-Bühl[3], Eric N Anderson[1], Brunhilde Wirth[3], Amit Singh[2], Deepa S Rajan[11]Pediatrics, Children's Hospital of Pittsburgh of UPMC, [2]University of Dayton, [3]University of Colone

GEMIN5 is essential for core assembly of small nuclear Ribonucleoproteins (snRNPs), the building blocks of spliceosome formation. Loss-of-function mutations in GEMIN5 lead to a neurodevelopmental syndrome among patients presenting with developmental delay, motor dysfunction, and cerebellar atrophy by perturbing snRNP complex protein expression and assembly. Currently, molecular determinants of GEMIN5-mediated disease have yet to be explored. While doing a genetic screen in Drosophila, we identified SMN as a suppressor of GEMIN5-mediated neurotoxicity in vivo. We discovered that an increase in SMN expression by either SMN gene therapy replacement or the antisense oligonucleotide (ASO) Nusinsersen, significantly upregulated the endogenous levels of GEMIN5 in mammalian cells and mutant GEMIN5 derived iPSC neurons. Further, we identified a strong functional association between the expression patterns of SMN and GEMIN5 in patient Spinal Muscular Atrophy (SMA) derived motor neurons harboring loss of function mutations in the SMN gene. Interestingly, SMN binds to the C-terminus of GEMIN5 and regulates GEMIN5 expression through the Tudor domain. Lastly, we show that SMN upregulation ameliorates defective snRNP biogenesis and alternative splicing defects caused by loss of GEMIN5 in iPSC neurons and in vivo. Collectively, these studies indicate that SMN is a potent regulator of GEMIN5 expression and neuropathologies.

1019S **The P3 Peptide Has Similar but Less Severe Effects than the Alzheimer's-associated Peptide Aβ(1-42) on Drosophila Longevity, Behavior, Neurodegeneration, and Gene Expression** Alfredo S Rojas Moreno, Zhiying Qiu, Amit A James, Andrés Nuño, Amelia M DeAndreis, Jacquelene A Hundelt, Marisa Fujimoto, Yuhao Pan, Joey Wong, Jeremy LeeDepartment of Molecular, Cell and Developmental Biology, University of California, Santa Cruz

Alzheimer's disease (AD) is a progressive neurodegenerative disease and is the most common cause of dementia. Progression of the disease results in memory decline and changes in behavior and personality. The main pathologies of AD are the presence of amyloid plaques and neurofibrillary tangles. The plaques are formed through the aggregation of amyloid beta (Aβ) peptide, a product of cleavage of the Amyloid Precursor Protein (APP). In one cleavage pathway, APP is

cleaved first by β-secretase and then ɣ-secretase, resulting in the production of "full-length" Aβ peptides, including Aβ(1-42), which is associated with AD. An alternative pathway uses α-secretase instead of β-secretase, generating a peptide called P3 (Aβ 17-42) that is 16 amino acids shorter than Aβ(1-42) and composed of the hydrophobic portion of Aβ(1-42). While P3 is found in the brains of AD patients, it is not known whether it is a contributor to Aβ aggregation or to AD progression.

Previously, we have shown that pan-neuronal expression of human P3 in Drosophila, using the APPL-Gal4 driver, exacerbates Aβ(1-42)'s deleterious effects. In addition, when P3 is expressed alone, it has similar but less severe neurotoxic effects than Aβ(1-42), as measured in RING (climbing) assays and longevity assays. In addition, electron micrograph analyses of flies expressing Aβ(1-42), P3, or both, using the GMR-Gal4 driver, showed that P3 had similar degenerative effects as Aβ(1-42) and exacerbated the effects of Aβ(1-42) when co-expressed with Aβ(1-42).

Recently, we have conducted RNA sequencing analysis with these flies. Our results indicate that pan-neuronal expression of P3 induces misregulation of many of the same genes that are misregulated when Aβ(1-42) is expressed. A large number of the misregulated genes encode proteins involved in proteolysis. Future experiments will focus on a small number of these genes to more thoroughly investigate the consequences of this misregulation.

Finally, since P3 is composed of 26 of the 42 amino acids of Aβ(1-42), we hypothesize that P3 interacts with Aβ(1-42). We are conducting co-immunoprecipitation experiments to determine whether P3 interacts directly with, or is part of a complex with Aβ(1-42). These results, coupled with our results showing that P3 has similar effects as Aβ(1-42) on longevity, behavior, degeneration, and gene expression, will allow us to determine whether P3 might be an important contributor to Aβ aggregation and to AD pathology.

1020S **Determining the Synthetic Lethality of Cancer-Related Mutations with Methyl and Ethyl Parabens in *Drosophila melanogaster*** Mikesha Carter[1], Stephanie Awuzie[21]San Francisco State University, [2]Biology, San Francisco State University

In society, there has been an increase in incidences of cancer due to increased exposure to chemicals and environmental toxins. Parabens, a family of small molecule preservatives, are found in over 85% of personal care products, foods, and the environment. However, it is unknown how exposure to parabens affects organisms with cancer-related mutations. Parabens act as xenoestrogens that accumulate in the human body over time and a recent study found that 99% of breast cancer tumor tissues sampled tested positive for parabens. The aim of our research is to determine the synthetic lethality of methyl and ethylparaben on *Drosophila melanogaster* with loss-of-function mutations in tumor suppressor genes. Our hypothesis is that increasing levels of exposure to methyl and ethylparabens will result in decreased survivability for *Drosophila melanogaster* with cancer-related mutations. Here, we are analyzing two specific tumor suppressor genes BRCA2 and P53. BRCA2 is a tumor suppressor gene that are associated with increased risk of breast, ovarian, and fallopian tube cancers. P53 is also, a tumor suppressor gene that prevents the over-proliferation of cells and are found in over 60% of all human cancer cases. To study the survivability of the *Drosophila melanogaster* with cancer-related mutations, we exposed P53 and

BRCA2 mutant larvae to varying concentrations of the drug. We added 2000, 3000, 4000, and 5000 mg/l of methylparabens to fly media vials and measured survivability by counting pupation and hatch. Overall, it was found that there was not an increased synthetic lethality in Drosophila with loss-of-function mutations in BRCA2 and p53 genes when exposed to parabens. Our future directions are to determine if increasing levels of exposure to parabens will result in an increase in DNA damage, decrease in fertility, and change in generational survivability in *Drosophila melanogaster* with cancer-related mutations.

1021S **Exploring the mechanistic roles of APC in the β-catenin destruction complex** Julia Kiefer, Khoi Le, Glorimar Jaramillo, David RobertsFranklin & Marshall College

The tumor suppressor, Adenomatous Polyposis Coli (APC), is an essential negative regulator of the Wg/Wnt signaling pathway and is inactivated in nearly 80% of all colon cancer cases. APC regulates Wg/Wnt signaling by participating in the "destruction complex" that targets the proto-oncogene, Armadillo/β-catenin (Arm/β-cat), for ubiquitin-mediated proteolysis. Despite nearly 30 years of research on APC, its precise mechanistic role in the destruction complex remains unknown. APC contains several Arm/β-cat binding sites that prior research from several groups has suggested play important mechanistic roles. We have been testing the importance of APC's β-cat-binding sites using a structure/function approach in Drosophila. Surprisingly, our previous research demonstrated that Arm/β-cat binding sites in APC2 are dispensable for Arm/β-cat destruction in cells of the embryonic epidermis that do not receive Wg/Wnt signaling, but are required in cells that receive Wg/Wnt signaling. These findings suggest that APC's β-cat binding sites are dispensable for basal Arm/β-cat destruction, but may have a unique mechanistic role(s) in cells that receive Wg/Wnt signal. We hypothesize that for basal Arm/β-cat destruction, Axin's β-cat-binding site may be compensatory and that APC and Axin β-cat binding sites are redundant. To test this hypothesis, we have generated APC and Axin transgenes either containing or lacking Arm/β-cat binding sites and are assessing the different transgene combinations for function in Drosophila embryos. Additionally, we are testing if APC's β-cat bindings sites function simply to recruit Arm/β-cat into the destruction complex by generating APC2 transgenes that replace the Arm/β-cat bindings sites with Arm/β-cat binding sites from other Arm/β-cat interacting proteins such as α-catenin, TCF, and Axin. To date, our results indicate that APC's Arm/β-cat binding sites are partially replaceable by Arm/β-cat binding sites from TCF and Axin, but not the Arm/β-cat binding site from α-catenin. Collectively, these findings suggest that APC's Arm/β-cat binding sites do not function simply to recruit Arm/β-cat into the destruction complex, but rather they also likely play a more complex mechanistic role(s). Furthermore, it suggests that specific contacts to Arm/β-cat may be required for efficient Arm/β-cat destruction.

1022S **Effects of Lactate dehydrogenase A (LDH-A) knockdown in *Drosophila* model of Alzheimer's disease** Fang Ju Lin[1], Andres Castillo[2], Trevor Stevens[2], Rachel Mazzeo[21]biology, Coastal Carolina University, [2]Coastal Carolina University

Human Alzheimer's disease (AD) is the most prevalent and lethal neurodegenerative disease. Memory loss and motor dysfunction are accompanied by pathological hallmarks like neurofibrillary tangles or amyloid plaques. In this study, we used Gal4-UAS system to drive the expression of human Amyloid beta 42 gene in *Drosophila* as model for Alzheimer›s disease, and the toxicity of amyloid plaques was evaluated in comparison to a UAS-GFP control. We found that AD flies expressed higher transcriptional level of LDH-A, and exhibited shorter lifespan and impaired locomotor function. Knockdown of LDH-A using RNAi rescued the disease phenotype in AD flies. As LDH-A is one of the conserved enzyme isoforms that could convert pyruvate to L-lactate, the increase of LDH-A in AD flies coincides with the higher level of lactate found in Alzheimer›s patients. In addition, we will use courtship suppression assay to assess whether learning and memory deficits in AD flies can also be rescued by the LDH-A knockdown.

1023S ***Drosophila ifc/degs1* mutants as a model for uncovering the pathogenesis of hypomyelinating leukodystrophy 18 (HLD18) in human** Yuqing Zhu[1], Haluk Lacin[1], Kevin Cho[2], Yi Zhu[1], Beth Wilson[1], Gary Patti[2], James B Skeath[11]Department of Genetics, Washington University School of Medicine, [2]Department of Chemistry, Washington University in St. Louis

Hypomyelinating leukodystrophy defines a heterogenous group of genetic disease characterized by failure of healthy

myelination in the central nervous system (CNS) during development. Next-generation sequencing has uncovered over 20 genes that cause a wide range of clinical manifestations with varying severity. Unexpectedly, most causal genes do not encode structural myelin proteins, suggesting indirect pathological mechanisms that result in low myelin levels in most human hypomyelinating leukodystrophies. Here, we present *infertile crescent* (*ifc*) mutants in *Drosophila* as a model to study the pathogenesis of HLD18, a recently discovered hypomyelinating leukodystrophy in human. *ifc*, or *degs1* in human, encodes a sphingolipid delta-4 desaturase that converts dihydroceramide (DhCer) to ceramide (Cer) in the final step of Cer *de novo* synthesis. *ifc*/*degs1* is highly conserved from fly to human, and we have generated *ifc* mutants with single nucleotide mutations that are found in similar regions to known human mutations in *degs1* mutants that cause HLD18. *ifc* is predominantly expressed in glial cells of the *Drosophila* nervous system. Homozygous mutant *ifc* larvae exhibit reduced brain volume, axonal swelling, and an elongated nerve cord. Notably, essentially all glial cell types manifest gross defects in morphology, such as bloating and mass accumulation of internal, likely ER, membranes, likely contributing to an observed defect in the ability of glial cells to extend their sheaths to fully around their neuronal cell body, axonal, and dendritic targets. On the metabolic scale, loss of *ifc* causes global disruption of lipid metabolism, DhCer accumulation, depletion of glial-specific lipid storage, and reduction in energy production in the CNS. Our work supports a model in which failure to convert DhCer to Cer results in the retention of excessive levels of DhCer in the ER, inappropriate accumulation of internal cell membranes, and defective glial sheath extension.

1024S **Yki/YAP/TAZ transcriptional co-activators drive neuro-glial stem/progenitor cell identity in high grade gliomas** Renee Read, Krishanthan Vigneswaran, Se-Yeong Oh, Nathaniel Boyd, William Read, Jeffrey OlsonEmory University School of Medicine

High-grade gliomas (HGGs), neoplasms derived from neuro-glial stem/progenitor cells, are the most common and lethal malignant primary brain tumors diagnosed in adults and children, with a median survival time of 14 months with current treatments. HGG tumorigenicity is often driven by genetic aberrations in receptor tyrosine kinases (RTKs), such as EGFR, and the Pi-3 kinase (PI3K) signaling pathway. Using *Drosophila*, mouse, and human HGG model systems of RTK-PI3K mutant HGGs, we identified the Yki/YAP and TAZ transcription co-activators, which regulate gene expression via Sd/TEAD co-factors, as key drivers of tumorigenicity downstream of oncogenic RTK signaling. Yki/YAP/TAZ are highly expressed in RTK-mutant human HGG cells, and regulate Sd/TEAD-mediated transcription of several transcription factors, neurodevelopmental genes, and receptors, including the EGFR RTK, that together create a feedforward loop with RTKs to maintain a neuro-glial stem/progenitor cell fate in HGG cells. Inhibition of Yki/YAP/TAZ in self-renewing stem-cell-like RTK mutant HGG cells can inhibit proliferation, block stem/progenitor cell fate, and elicit apoptosis. In particular, the benzoporphyrin derivative verteporfin, a disruptor of YAP/TAZ-TEAD mediated transcription, specifically induced apoptosis of cultured patient-derived HGG cells, suppressed expression of YAP/TAZ transcriptional targets, including RTKs, and conferred significant survival benefit in an orthotopic xenograft HGG model. Our efforts led us to initiate phase 0 and phase 1/2 clinical trials of Visudyne, an FDA-approved liposomal formulation of verteporfin, in which we have verified verteporfin uptake into tumor cells and are now testing the therapeutic efficacy of verteporfin in human patients. Finally, we have examined mechanisms of response and resistance to verteporfin, and we have evidence that stem-like HGG cells can escape the effects of YAP/TAZ inhibition by recapitulating neuro-glial developmental cell fate transitions. Together, our data indicate that targeting YAP/TAZ functionality is a promising therapeutic approach for HGG.

1025S **Disrupted endoplasmic reticulum-mediated autophagosomal biogenesis in a *Drosophila* model of C9-ALS/FTD** Hyun Sung, Elaine L Yang, Thomas E LloydJohns Hopkins University

Autophagy is a major cellular pathway for the clearance of protein aggregates and damaged organelles, and multiple intracellular organelles participate in the process of autophagosomal development from membrane formation to organelle maturation and content degradation. Dysregulation of the autophagy pathway has been implicated in the pathogenesis of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), however the mechanisms underlying autophagy impairment in these diseases are not fully understood. Since the expansion of GGGGCC (G_4C_2) repeats in *C9orf72* is the most common inherited cause of both ALS and FTD (C9-ALS/FTD), we investigated the autophagy pathway in *Drosophila* motor neurons expressing 30 repeats of G_4C_2 (30R). We analyzed the dynamics of autophagosomes *in vivo* and found that 30R expression is linked to a marked reduction in the number of terminal autophagosomes, which results in the attenuation of autophagosomal trafficking in axons while the remaining autophagosomes undergo normal maturation during their retrograde axonal transport. 30R expression also impairs endoplasmic reticulum (ER) integrity, leading to elevated ER stress and altered ER structure and dynamics. However, the inhibition of ER stress in 30R-expressing motor neurons restores neither ER structure/dynamics nor autophagosomal number, suggesting a neuronal autophagy pathway independent of ER stress. Importantly, we

found that autophagosomes are generated from dynamic ER tubules at motor synaptic boutons, and this process fails to occur in animals expressing 30R. Our results thus indicate that pathogenic G_4C_2 hexanucleotide repeats impair autophagy in motor neurons by affecting intracellular organelle dynamics, specifically the ER dynamics involved in the autophagosomal biogenesis required for maintaining neuronal proteostasis.

1026S **Biallelic missense variants in *OGDH* encoding oxoglutarate dehydrogenase lead to a neurodevelopmental disorder characterised by global developmental delay, movement disorder and metabolic abnormalities** Ella Whittle[1], Madison Chilian[2], Ehsan Ghayoor Karimiani[1], Helga Progri[2], Daniela Buhas[3], Melis Kose[4], Rebecca D. Ganetzky[4], Mehran Beiraghi Toosi[5], Paria Najarzadeh Torbati[6], Reza Shervin Badv[7], Ivan Shelihan[8], Hui Yang[9], Houda Zghal Elloumi[9], Sukyeong Lee[10], Yalda Jamshidi[1], Alan M. Pittman[1], Henry Houlden[11], Erika Ignatius[12], Shamima Rahman[13], Reza Maroofian[1], Wan Hee Yoon[2], Christopher J. Carroll[11]St. George's, University of London, [2]Oklahoma Medical Research Foundation, [3]McGill University, [4]Children's Hospital of Philadelphia, [5]Mashhad University of Medical Sciences, [6]Next Generation Genetic polyclinic, [7]Tehran University of Medical Sciences, [8]McGill University Health Centre, [9]GeneDx, [10]Baylor College of Medicine, [11]UCL Institute of Neurology, [12]University of Helsinki and Helsinki University Hospital, [13]Great Ormond Street Hospital for Children NHS Foundation Trust

The *2-oxoglutarate dehydrogenase* (*OGDH*) encodes an E1 subunit of a-ketoglutarate dehydrogenase complex (a-KGDHc) in the Krebs cycle that plays an essential role in cellular metabolism. Here, we performed a detailed clinical characterisation of four unrelated individuals from consanguineous families carrying biallelic variants in *OGDH* with a neurodevelopmental disorder. The individuals presented with global developmental delay, movement disorder and metabolic abnormalities. In all four individuals, we identified three homozygous missense variants in *OGDH*. In-silico homology modelling predicts that p.Pro189Leu and p.Ser297Tyr variants interfere with the structure and function of OGDH. Fibroblasts from individual 1 showed that the p.Ser297Tyr variant led to a higher degradation rate for the OGDH protein. OGDH protein with p.Pro189Leu or p.Ser297Tyr variants in HEK293 cells showed significantly lower protein levels compared to wild-type protein. Furthermore, we showed that expression of *Drosophila Ogdh* (*dOgdh*) carrying the homologous mutations to the p.Pro189Leu or p.Ser297Tyr failed to rescue developmental lethality caused by loss of *dOgdh*. SpliceAI, the variant splice predictor, predicted that the c.935G>A; p.Arg312Lys impacts slicing, which was confirmed by a mini-gene assay in HEK293 cells. Hence, we established that biallelic variants in *OGDH* are a cause of childhood-onset neurodevelopmental disease.

1027S **Traumatic brain injury (TBI)-induced metabolic dysfunction and response to dietary restriction in female fruit flies** Hannah M Turnage, Rebecca N Ray, Rebecca DelventhalBiology, Lake Forest College

Traumatic brain injuries (TBI) occur when sudden trauma to the head causes damage to the brain. This can lead to long-term physical and cognitive problems. Many distinguishing features of a TBI in humans also occur in fruit flies, making them an ideal model to study the long-term effects of TBIs. Previous research on male fruit flies has shown that TBI decreases lifespan, locomotor ability, and causes metabolic dysfunction. Considering that female fruit flies are known to have different metabolic regulation due to egg production and ability to store more fat, we wanted to ask if females display similar metabolic dysfunction to males following TBI? We delivered a TBI using a High-Impact Trauma (HIT) device (Katzenberger et al., 2013) to 1 week old *Canton-S* females. Females displayed higher acute mortality, meaning a higher percent of females die within 48 hours, than males who received the same injury. We also measured starvation survival time at 48hr, 2wk, and 4wk post-TBI to see if females also displayed a pattern of TBI-induced starvation sensitivity similar to males. While this work is ongoing, initial results appear to show uninjured female flies survived longer than the TBI females at 48hr and 2wk post-TBI, similar to what has previously been observed in males. Further work to examine whether females also display a similar decrease in lipid stores in response to TBI as males is currently being undertaken. Previous research in males has also shown that dietary restriction (DR) ameliorates the TBI-induced decline in lifespan and locomotor ability. Considering female flies are also known to be more responsive to DR, we asked does DR mediate the effect TBI has on lifespan and climbing ability in females to a greater degree? Female flies were placed on normal diet (6% yeast) or low protein diet (1% yeast) post-eclosion, then given TBI at ~1 week of age. DR extended the lifespan for TBI and uninjured females to a greater magnitude than in males. We also measured climbing ability of female flies, TBI and uninjured, on normal or DR food at 48hr, 2wk, and 4wk post-TBI. Female flies with TBI on DR climbed significantly better than flies with TBI on normal food at 2wk post-TBI, but not at 48hr or 4wk, similar to males but at a higher magnitude. In future studies we will examine the minimum length of DR treatment required to observe benefit, how mating status affects TBI-induced metabolic dysfunction in females, and underlying sex-specific molecular pathways.

1028S **Functional contribution of Scully and its interacting molecules to dementia** Carolyne Chepkosgei, Maya

Solis, Paul Rafael Sabandal, Kyung-An HanThe University of Texas at El Paso

Alzheimer's disease and related dementias (ADRD) are neurodegenerative diseases characterized by progressive deterioration of cognitive functions. Many genetic and non-genetic (e.g., age and social stress) risk factors are associated with ADRD. However, whether and how these risk factors interact for ADRD initiation and progression remain unclear. We conducted an unbiased genetic screen for novel ADRD genes and identified Scully (*Scu*) as a genetic risk factor interacting with social stress for dementia. *Scu* is a multifunctional mitochondrial enzyme and is known to bind beta amyloid peptides. The heterozygous *Scu* flies (*Scu/+*) exhibited the accelerated loss of memory and inhibitory control in an aging-dependent manner. To identify the specific cellular pathway by which *Scu* contributes to dementia, we investigated the molecules known to physically or genetically interact with *Scu* (SIMs for *Scu* interacting molecules). We generated the flies transheterozygous for *Scu* and individual SIMs (15 out of total 59 SIMs), and assessed their inhibitory control capacities at the ages of 4 days and 2 weeks. At 4-days old, all *Scu*/SIM transheterozygous lines showed normal inhibitory control. At 2 weeks old, however, two transheterozygous lines, *Scu/+;;CG8036/+ and Scu/+;Cndp2/+*, exhibited dysfunctional inhibitory control. CG8036 is a transketolase important for glycolysis and Cndp2 (cytosolic non-specific dipeptidase 2) hydrolyses dipeptides into amino acids. The progress of this study will be presented. The findings of our study will fill the knowledge gap on the cellular pathways leading to ADRD.

1029S **Using *Drosophila* as a Model to Study Environmental Toxicity Relevant to Parkinson's Disease** Angeline Claudia Atheby[1], Lindsey Ruggiero[2], Hakeem Lawal[21]Biological Sciences, Delaware State University, [2]Delaware State University

Parkinson's Disease (PD) is the second most common neurodegenerative disease characterized in part by the loss of dopaminergic neurons in the substantia nigra pars compacta. Epidemiological studies have established that exposure to certain pesticides and herbicides increase an individual's likelihood of developing PD. Although the full catalog of pesticides that pose risks for PD is not currently known, some potentially toxic compounds are commonly used for agricultural or gardening purposes, and may be purchased at local stores. The objective of this study therefore, was to determine whether different combinations of these commercially-available pesticides and herbicides have either a synergistic or additive effect on toxicity in *Drosophila*. We first established a dose response curve in the following pesticides: acephate, atrazine, and diuron. We then exposed *Drosophila* to different combinations of the compounds at a concentration at which each alone was minimally toxic. We performed survival and locomotion ability analyses on each experimental group. Here, we report that combinations involving atrazine and diuron show strong decreases in survival that were greater than each single pesticide alone. We also present data on our analysis of the effects of these compounds on reactive oxygen species generation and measures of neurotoxicity. Together, our data suggest that exposure to combinations of commonly-used pesticides such as atrazine and diuron may elevate susceptibility to environment toxins.

1030S **Assessing the roles of *BUD13* in rare human genetic diseases** Mikiko Oka[1,2], Oguz Kanca[1,3], Yi-An Ko[4], Giorgio Sirugo[4,5], Daniel J Rader[4,6], Michael F Wangler[1,3], Hugo J Bellen[1,3], Shinya Yamamoto[1,31]Molecular and Human Genetics, Baylor College of Medicine, [2]Jan & Duncan Neurological Research Institute, Texas Children's Hospital, [3]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, [4]Division of Translational Medicine and Human Genetics, Perelman School of Medicine, University of Pennsylvania, [5]Institute of Systems Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, [6]Department of Genetics, Perelman School of Medicine, University of Pennsylvania

Pre-messenger RNA (mRNA) splicing is a critical process and mutations in spliceosome genes cause numerous diseases that affect in multiple organs. The REtention and Splicing (RES) complex, which consists of three different proteins (BUD13, SNIP1, and RBMX2), is involved in the splicing of short introns with high GC contents. A recent clinical study has identified multiple patients with homozygous variants in *BUD13* who present with a developmental disorder with lipodystrophy and progeroid features. In addition, through the efforts of the Undiagnosed Diseases Network (UDN), we identified a patient with rare compound heterozygous variants in *BUD13* in a patient who exhibits distinct musculoskeletal symptoms. However, how these variants affect the function of *BUD13* and whether it contributes to these phenotypes are unclear.

To determine how variants in *BUD13* affect protein function, we generated transgenic fly strains that express the human reference or variant human *BUD13* cDNA using the UAS/GAL4 system. We found that ubiquitous overexpression of reference *BUD13* causes lethality whereas wing-specific overexpression causes morphological defects. In contrast, two

of the *BUD13* variants tested so do not induce these phenotypes, suggesting that they are loss of function variants. To further explore the biological function of *BUD13* and determine its expression pattern in *Drosophila*, we generated a knock-out/knock-in allele which replaces the coding region of *CG13625*, the fly *BUD13*ortholog, with *a Kozak-GAL4* cassette. *CG13625* is an essential gene. We are now in a position to determine the precise expression pattern and functional consequences of the *BUD13* variants and determine the *in vivo* roles of the RES complex.

1031V **The epigenetic modifier EHMT/G9a regulates sleep via its role in metabolism-relevant cellular substrates** Mireia Coll-Tané[1], Lara V van Renssen[1], Boyd van Reijmersdal[1], Marieke Klein[1], Franziska Kampshoff[1], Chiara Pignato[1], Rik Scharn[1], Human Riahi[1], Naihua N Gong[2], Matthew S Kayser[2], Tjitske Kleefstra[1], Annette Schenck[11]Human Genetics, Radboud University Medical Center, [2]University of Pennsylvania

Sleep disturbances are common in neurodevelopmental disorders and represent a major burden for affected individuals and their families, yet they remain largely uncharacterized. Here, we show that individuals with loss-of-function mutations in the epigenetic regulator *EHMT1*, diagnosed with Kleefstra syndrome, suffer from specific sleep disturbances. Moreover, we show that common variants in the same gene are also associated with multiple sleep traits. We were able to recapitulate these defects in *Drosophila* mutants affecting the *EHMT1* orthologue, *G9a*. We show that this role maps to both insulin-producing cells and the fat body, revealing that G9a is required in these metabolically relevant cellular substrates for sleep integrity. Next, we show that G9a regulates sleep through multiple and distinct downstream targets in insulin-producing cells and the fat body. Metabolomics analysis in *G9a* mutants further revealed a broad metabolic deregulation; particularly, we found the methionine metabolism pathway to be greatly deregulated. *The G9a* mutants showed a significant increase in methionine sulfoxide (Met-SO) levels, the product of methionine oxidation by reactive oxygen species (ROS) and a key player in ROS detection and cellular redox homeostasis. Knockdown of the enzyme responsible for Met-SO reduction, *Methionine sulfoxide reductase A*, in insulin-producing cells but not in the fat body recapitulated the sleep disturbances of *G9a* mutants. Last, we show that G9a's sleep fragmentation can be rescued in adulthood using a translational behavioral regime based on human sleep-restriction therapy. Our work provides novel fundamental insights into the pathophysiology of sleep disturbances in Kleefstra syndrome and explores new approaches toward their treatment.

1032V **The microRNA miR-33 regulates mutant TDP-43 toxicity in transgenic flies** Swapnil Pandey, Alfonso Martin Pena, Deepak Chhangani, Diego E Rincon-LimasNeurology, 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.

1033V **Tumour-induced muscle wasting is mediated by mitochondrial β-oxidation in a *Drosophila* model of cancer cachexia** Callum Dark[1,2], Nashia Ali[1,2], Sofya Golenkina[1,2], Daniel Bakopoulos[1,2], Ben Parker[2], Tarosi Senapati[3], Sean Millard[3], Louise Cheng[1,21]Peter MacCallum Cancer Centre, [2]Anatomy and Physiology, University of Melbourne, [3]School of

Biomedical Sciences, University of Queensland

Cancer cachexia is a tumour-induced wasting syndrome seen in approximately 80% of late-stage cancer patients, characterised by the loss of skeletal muscle and adipose tissues. Despite this, there is currently no gold-standard of treatment, and a lack of diagnostic biomarkers. We have recently established a *Drosophila* larval model of cancer cachexia (PMID 34473940), which recapitulates muscle and fat wasting observed in patients. Using this model, we found that prior to overt muscle wasting, muscles exhibited a downregulation of insulin signalling through nuclear translocation of FOXO, mediated by tumour secreted ImpL2. This in turn led to an increase in mitochondria fusion and a reduction in mitochondria function. Interestingly, we also found that cachectic muscles switched from utilisation of glycogen to lipids prior to muscle wasting, resulting in a depletion of glycogen, and then lipid stores. Mitochondrial β-oxidation is the major metabolic process which catabolizes fatty acids, which produces the primary energy source for various cellular processes. Proteomics analysis showed that cachectic muscles exhibited an upregulation of several regulators of β-oxidation, including CPT1/withered (whd). Upon muscle-specific knockdown of FOXO, whd, or mitochondria fusion regulator marf, muscle integrity was restored in tumour-bearing animals. We further demonstrate the interplay between mitochondria fusion, lipid metabolism and FOXO in regulating muscle integrity in the presence of the tumour. Finally, we found that supplementing the diet with nicotinamide or lipids, further attenuated tumour induced muscle wasting, offering potential treatment options for cachexia.

1034V **Multiple pre-cachexic changes occur in larval muscles** Mardelle Atkins, Ellen Thompson, Oluwapelumi Soyebo, Grace StegemollerBiological Sciences, Sam Houston State University

Cachexia is a wasting syndrome common in late-stage cancer patients. Its symptoms include rapid weight loss, metabolic alterations, and muscle weakness/loss. To study cachexic wating effects on muscles, our lab utilizes a *Drosophila melanogaster* tumor model which displays the syndrome's major symptoms. In this model, larvae fail to pupate and display observable physical wasting of fat and muscle from 8-12 days AEL. We identified that functional and structural failure of muscles begin at approximately D10 AEL. We are now exploring earlier changes in the muscles to discover the etiology of muscle failure and wasting. We find that Myosin heavy chain mislocalization precedes gross muscle defects and that it correlates with changes in nuclei and other organelles. This work identifies multiple, reproducible changes which precede muscle fiber wasting and loss. These results may provide valuable insights into the origins of muscle cachexia.

1035V **Identification of Candidate Genetic Modifiers in a *Drosophila melanogaster* Model of *ARID1B*-Related Coffin-Siris Syndrome** Rebecca A MacPherson, Sarah C Macon, Katelynne M Collins, Robert R H Anholt, Trudy F C MackayCenter for Human Genetics and Department of Genetics and Biochemistry, Clemson University

ARID1B encodes a subunit of the *Brahma-Related Gene-1* Associated Factor (BAF) complex, which, in addition to chromatin remodeling, is critical for cellular differentiation and regulation of gene expression. Mutations in *ARID1B* are associated with autism spectrum disorder, a disorder of unknown etiology associated with intellectual disability and/or changes in language and social behavior, and Coffin-Siris syndrome (CSS), a rare, highly variable disorder of chromatin modification associated with intellectual disability, digit anomalies, seizures, and hypotonia. We hypothesize that this phenotypic variability may be explained by naturally segregating genetic modifiers, although identification of such modifiers in human populations is challenging for rare disorders due to insufficient statistical power. We can use *Drosophila melanogaster* as a model system to identify candidate genetic modifiers for *ARID1B*-related CSS, as *ARID1B* is highly conserved and flies can be economically reared in large numbers within a controlled environment. Furthermore, the *Drosophila melanogaster* Genetic Reference Panel (DGRP), a panel of naturally derived, fully sequenced, inbred lines with naturally occurring molecular genetic variation, can be used to perform genome-wide associated studies (GWAS). Previously, we used the bipartite *UAS-GAL4* system to generate RNA interference fly models of CSS with ubiquitous knockdown of *osa*, the fly ortholog of *ARID1B*. Here, we have also generated CSS models with wing-specific knockdown of *osa*. In small-scale studies, we crossed these ubiquitous and tissue-specific fly models to 10 DGRP lines and found background-dependent changes in phenotype (night sleep and wing vein development, respectively). We then crossed the wing-specific model of *ARID1B*-CSS to ~400 DGRP lines and performed a GWAS to identify candidate genetic modifiers, and will present the results of this study.

1036V **Mortality Index and Assessment of Motor Function of Wild- Type Drosophila melanogaster, following multiple TBI events, utilizing the H.I.T. Device** Megan N Ashworth, Kimberly RowlandLynn University

Traumatic brain injuries (TBI) are the leading cause of neurological deficits and mortality worldwide (Masel BE, DeWitt

DS, 2010). This disease process induces a cascade of molecular events that lead to cell damage and death in the brain. The primary and secondary injuries that ensue after a TBI can have detrimental effects on motor function (Katzenberger et al., 2013). To investigate TBI's effect on motor function, we utilized the mechanical high-impact trauma (HIT) device, using a Drosophila melanogaster model (Katzenberger et al., 2013). The flies were exposed to high force, acceleration, and deceleration, similar to TBI events in humans. They were also subjected to multiple hits with a five-minute rest period in between each hit. Flies subjected to TBI exposure experienced temporary ataxia and incapacitation. Most flies regained motor function following a five-minute rest period between hits. We also analyzed the mortality index to observe if there is a correlation between the number of traumatic brain injuries and death. Our data indicate no significant correlation between the number of hits and death, indicating that there are other molecular mechanisms involved that play a role in death following TBI. Furthermore, we will analyze the locomotor function of the flies following TBI. We will perform climbing assays, following TBI, utilizing the Drosophila Activity Monitor device (DAM) to assess how traumatic brain injuries disrupt motor function in flies.

1037V **Insights into mitochondrial dynamics of familial Parkinson's Disease (PD)** Sonia Narwal[1], SONIA NARWAL[2], Meghana Tare[2], Amit Singh[31]Biological Sciences, BITS-Pilani, Pilani Campus, [2]Biological Sciences, BITS-Pilani, Pilani, [3]Department of Biology, University of Dayton

Selective dopaminergic neurodegeneration and presence of Lewy Bodies, which are aggregation of incorrectly folded α-Synuclein, are major pathological hallmarks of Parkinson's disease. Another form of PD is characterized by loss-of-function of *parkin*, which encodes an E3-ubiquitin ligase. However, molecular interaction between *parkin* and *α-Synuclein* is poorly explored. Additionally, impaired mitochondrial dynamics have been reported in PD, also due to mutations in *α-Synuclein* and *parkin*. Nonetheless, effect of *parkin*-downregulation due to α-Synuclein on mitochondrial dynamics is not much explored.

In this study using *Drosophila* model, we show that *α-Synuclein*-overexpression and *parkin*-downregulation cause progressive locomotory dysfunctions and reduced dopaminergic clusters; PPM1&2, PPL1 and PPM3 in posterior region of adult brain. *α-Synuclein*-overexpression and *parkin*-downregulation result into swollen mitochondrial morphology specifically in PPL1& PPM3. Further, we found that transcriptional downregulation of *parkin* is associated with accumulated *α-Synuclein* resulted in altered expression of genes involved in regulation of the mitochondrial dynamics. Although, *α-Synuclein*-overexpression with *parkin*-downregulation exhibits similar locomotory dysfunction and reduced dopaminergic neurons, the effects are less pronounced when compared to *parkin*-downregulation alone. We observed altered mitochondrial morphology in PPL1& PPM3 clusters. Our study will provide insights for a link between *α-Synuclein* and *parkin* which alter the mitochondrial dynamics in PD.

1038V **Investigating autophagy dysregulation in C9orf72-linked Amyloid Lateral Sclerosis and Frontal Temporal Dementia** Paulien Hermine Smeele[1], Thomas Vaccari[21]Univeristy of Milan, [2]University of Milan

G_4C_2 hexanucleotide repeat expansions in *C9orf72* are the most common genetic cause of Amyloid Lateral Sclerosis (ALS) and Frontal Temporal Dementia (FTD). The repeat RNA formed by the bidirectional transcription of the repeat expansions can aggregate into RNA foci and can be translated by RAN translation to form Dipeptide Repeat (DPR) proteins. Accumulating evidence points towards these repeat RNA and DPR species being major contributors of disease pathogenesis through gain of function mechanisms, such as disruption of the Nuclear Pore Complex (NPC). Indeed, initial evidence suggests that the nuclear translocation of TFEB – a master transcriptional regulator of the autophagy pathway – is compromised by G_4C_2 toxicity.

As TFEB is emerging as a promising pharmacological target for neurodegenerative diseases, we aimed to further characterize G_4C_2 -induced TFEB dysregulation *in vivo*, using well-characterized *Drosophila* models of G_4C_2 toxicity. In addition to performing subcellular analyses of Mitf (*Drosophila* TFEB) localization on larval tissues, we also attempted to closely recapitulate C9-ALS/FTD using the inducible GeneSwitch GAL4 system to drive G_4C_2 repeat expression only in adult neurons.

In line with previous observations indicating compromised nuclear import, we observe a reduction in nuclear localization of Mitf upon overexpression of G_4C_2 repeats as compared to controls. In addition, we show decreased *Mitf* mRNA expression, consistent with the fact that Mitf transcriptionally regulates itself. Surprisingly, Mitf appears to accumulate at the protein level; both the active, non-phosphorylated and the inactive, phosphorylated form. These results suggest that proteasomal degradation of Mitf or its mTOR-mediated phosphorylation may additionally be disrupted by G_4C_2 toxicity. We are further investigating these two possibilities and exploring genetic modulators of Mitf in the context of G_4C_2

toxicity.

Importantly, Snap29, a SNARE protein which mediates autophagosome-lysosome fusion in late stages of the autophagy pathway, appears to modulate Mitf regulation and localization both *per se* and upon expression of G_4C_2. Further, performing a genetic interaction in the adult fly eye, we show that reducing *Snap29* levels either by RNAi or by a heterozygous loss of function mutation, strongly suppresses G_4C_2 toxicity. We are currently dissecting how Snap29 may directly or indirectly regulate Mitf and whether this relates to the observed suppression of G_4C_2 toxicity.

1039V **Adult neurogenesis during brain metastasis in *Drosophila*** Yoichi Kawai[1], Erina Kuranaga[1], Masayuki Miura[2], Yuichiro Nakajima[21]Tohoku University, [2]Graduate School of Pharmaceutical Sciences, The University of Tokyo

Brain metastasis is one of the major causes for the high mortality rate of cancer patients where different cellular origins metastasize into brains. While brain metastasis is a common phenomenon associated with cancers, precisely how distinct tumor-host interactions lead to selective nature of metastasis in the specific complex organ remains elusive. Here, we establish an *in vivo* model to investigate tumor-host interactions underlying brain metastasis, using the *Drosophila* allograft-based tumorigenic assay. Transplanted larval brain tumors proliferate dramatically in the adult flies, which eventually cause host organismal death. During tumor progression, tumor cells spontaneously detach from the transplanted tumor and migrate into distant organs. Detached tumor cells frequently proliferate in the head, remodel the blood brain barrier and invade into the brain, reflecting cellular features of brain metastasis. From RNA-seq analyses, we further found up-regulation of mitosis-related genes in the host brain of tumor-allografted flies, and confirmed up-regulation of these genes and cell cycle progression in the adult brain, suggesting that adult neurogenesis occurs upon interactions with metastatic tumors. Strikingly, blocking of neurogenesis in host neural cells suppresses tumor progression including brain metastasis and systemic tumor proliferation. Together, these results indicate that metastatic tumor cells trigger adult neurogenesis in the host brain, which promotes further tumor progression by a positive feedback mechanism.

1040V **oxt mutants, developmental patterning and Desbuquois Dysplasia/Spondyloocular syndrome** Line A Hofmann[1,2], Kavita Arora[1], Rahul Warrior[11]Developmental & Cell Biology, University of California, Irvine, [2]BioVersys AG

The proteoglycan synthesis pathway is highly conserved from invertebrates to humans Addition of sugar chains of heparan and chondroitin sulfate proteoglycans (HSPGs and CSPGs) is initiated when the Oxt enzyme covalently attaches xylose to a serine residue on proteoglycan core proteins. Humans have two closely related orthologs XYLT1 and XYLT2 compared to the single fly *oxt* gene. Mutations in XYLT1 cause Desbuquois Dysplasia 2 (OMIM # **#** 615777) characterized by skeletal defects, short stature and extremities, deformed facial features, and intellectual disabilities. Mutations in XYLT2 are linked to Spondyloocular syndrome (OMIM **#** 605822) which has overlapping features as well as eye defects. We have generated mutations in *oxt* and characterized their effects on proteoglycan synthesis and developmental patterning. We have tested the ability of human XYLT1 and XYLT2 genes to rescue *oxt* mutants and discuss its implications for the requirement for proteoglycans in developmental patterning and human disease.

1041V **Toxicity of glyphosate based herbicide Roundup® on non-target organisms: A mechanistic insights using *Drosophila melanogaster* a model of human disease** Anathbandhu Chaudhuri[1], Ankur Chaudhuri[2], Kim Lackey[3], Britton O'Shield[3], Sibani Chakraborty[2], Natraj Krishnan[41]Biology, Stillman College, [2]Microbiology, West Bengal State University, [3]Biology, University of Alabama, [4]Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University

Glyphosate based herbicides (GBH) have serious negative impact on human health and non-target organisms but mechanistic understanding remains very limited. This study tested the toxicological effects of the commercial herbicide Roundup® (RUP) on stress tolerance, movement behavior and oxidative stress *in vivo* using *Drosophila melanogaster.* Cellular morphology and the physical and chemical properties of Drosophila Schneider (S2) cells were also tested to confirm the effect of GBH at the cellular level *in vitro*. *In silico* modeling study was conducted to ascertain the binding affinity of GBH to carbonyl reductase; a key enzyme regulates oxidative stress. Chronic exposures to commercially formulated concentrated RUP develop Parkinsonian symptoms and impaired stress tolerance capacity in adult flies. Fly activity and rhythmicity significantly diminished with a remarkable decrease in negative geotaxis ($P < 0.05$), irrespective of sex, within 24 hours of acute exposure to RUP. RUP down regulates *sniffer* mRNA expression with a significant reduction in carbonyl reductase enzyme activity thereby trigging the formation of protein carbonyl in fly tissues indicating RUP induced oxidative stress in fly. *In silico* modeling of glyphosate, its surfactant polyoxyethyleneamine (POEA) and RUP (glyphosate plus POEA combined) suggests that all the individual components including RUP have a significant binding

affinity to carbonyl reductase (CR) protein. Each of the interacting residues present at the binding site of CR was computationally mutated to alanine to evaluate individual residue contributions towards the interaction of glyphosate, POEA and its combination (RUP). We observed that RUP has a much higher affinity to CR compared to glyphosate and POEA alone. Flowcytometry analysis of S2 cells revealed that the RUP drastically changes the physical and chemical properties of the cells and possibly interferes with the cell cycle in a dose dependent manner. Two-dimensional dot plot analysis showed a significant shift of a majority of the cell populations with a drastic decline in DNA content on the herbicide exposed S2 cells. *In vitro* cytological studies also revealed that RUP caused a significant change in tubulin protein architecture and the shape of the nucleus in the S2 cells. Thus, we hypothesize that RUP cause movement disorders by triggering oxidative stress and possibly interfering with cell cycle regulation.

1042V **Behavioral sensitivity and toxicity of the anesthetic isoflurane in *Drosophila* is modulated by expression of *Ndi1*, a yeast single-subunit NADH dehydrogenase** Luke A Borchardt[1], Amanda R Sharenbrock[1], David A Wassarman[2], Misha Perouansky[11]Department of Anesthesiology, University of Wisconsin-Madison, [2]Department of Medical Genetics, University of Wisconsin-Madison

Mitochondrial mutations lead to the most common inherited disorders of metabolism. Leigh syndrome (LS), a severe neurodegenerative disease, is caused by mutations in Complex I (CI) of the mitochondrial electron transport chain (mETC). LS patients and animal models of LS are hypersensitive to volatile general anesthetics (VGAs) such as isoflurane and are at increased risk of perioperative complications. We used an LS model in *Drosophila* caused by a mutation in the mitochondrially-encoded *ND2* (mt:*ND2*^{del1}) subunit of CI to study anesthetic-induced neurotoxicity (AiN). At 11-13 days old, mt:*ND2*^{del1} flies exposed to 2% isoflurane for 2 hours exhibited significantly increased 24-hour mortality when compared to unexposed counterparts. This phenotype was age-dependent and was modulated by oxygen concentration. Other labs have shown that expression of the yeast gene *Ndi1*, which encodes a single-subunit NADH dehydrogenase, can rescue CI mutant phenotypes in flies. To further explore the interaction between mitochondrial disorders and anesthetics, we ubiquitously expressed *Ndi1* in both wild type and mt:*ND2*^{del1} mutant flies using the GeneSwitch drug-inducible expression system. Expression of *Ndi1* in wild type flies increased their behavioral half-maximal effective concentration (EC_{50}) of isoflurane, whereas expression of *Ndi1* in mt:*ND2*^{del1} mutant flies had no effect on the EC_{50}. This indicates that the mt:*ND2*^{del1} mutation interferes with the ability of *Ndi1* to modulate anesthetic sensitivity. To better understand the mechanism of AiN, we assayed mt:*ND2*^{del1}-*Ndi1* flies for their 24-hour mortality after exposure to isoflurane under normoxic and hyperoxic conditions. We found that *Ndi1* expression delayed the onset of susceptibility to AiN but did not prevent AiN susceptibility from developing at an older age. These data indicate that *Ndi1* expression modulates both anesthetic sensitivity and some effects of the mt:*ND2*^{del1} mutation but does not directly block the mechanistic cause of AiN. This work is part of an ongoing project aimed at determining the mechanisms underlying AiN.

1043T **A collection of flippase-dependent conditional inactivation and reactivation alleles of conserved genes in *Drosophila*** Ming Fa, Ying Tan, Graeme Mardon, Rui ChenGenetiVision Corporation

To complement existing resources, we are generating a collection of 2,800 conditional alleles that allow a specific gene to be turned off or on at any time in any cell type for a large fraction of the conserved genes in *Drosophila*. Through leveraging the existing large collection of transgenic "CRIMIC" stocks, a custom Flippase-dependent conditional activation/inactivation cassette (termed "*flip-flop-loxP*") is inserted into an intron between two early coding exons. The *flip-flop-loxP* cassette permits conditional gene inactivation or reactivation controlled by Flippase expression. Therefore, the resulting collection is distinct from existing resources and allows mitosis-independent recombination, enabling mosaic analysis of gene function during different development stages and in adults. Moreover, this method allows restoration of gene function with full cell-type and temporal control. The *flip-flop-loxP* cassette is an improvement to the previously published *flip-flop* cassette with two *loxP* excision sites flanking the EGFP reporter such that expression of Cre recombinase can remove the EGFP tag in the event that the function of the EGFP fusion protein is compromised. The efficacy of this strategy has been validated for both gene expression and function. To maximize the usage of this new collection, we invite the research community to nominate genes of their choice for conversion from CRIMIC to *flip-flop-loxP* alleles. Once generated, this collection of conditional alleles will complement existing resources by adding significant capability to investigate gene function in any tissue/cell type in developing or adult flies by either knockout or restoration of function at any desired time, including in non-mitotic cells. This added flexibility of gene manipulation addresses critical gaps in current collections and allows researchers to probe key questions by separating gene function during development from adult stages, further expanding the powerful set of genetic tools available in *Drosophila*.

1044T **Fourth Chromosome Resource Project** Michael Stinchfield[1], Brandon Weasner[2], David Zhitomirsky[3], Bonnie

Weasner[2], Kevin Cook[2], Justin Kumar[2], Michael B O'Connor[3], Stuart Newfeld[11]School of Life Sciences, Arizona State Univ, [2]Biology, Indiana University, [3]Genetics, Cell Biology and Development, University MInnesota

The 4th chromosome is the final frontier for genetic analysis in Drosophila. Small and devoid of recombination the 4th has long been ignored. Nevertheless, it contains 79 protein coding genes. 74% have human homologs and 68% of homologs have a disease association. A complete understanding of metazoan biology requires the examination of 4th chromosome genes. To advance this effort, the 4th Chromosome Resource Project (FCRP) has deposited more than 250 strains at the Bloomington and Kyoto Stock Centers to date. Strains from our project are divided into five collections. First, we have adopted methods from the Gene Disruption Project as well as pioneered one ourselves. Employing MiMIC and CRIMIC conversion genetics, nearly all protein coding genes now have a loss of function mutant with a gene trap T2A.GAL4 and a protein trap with an eGFP tag. Images for each of these lines are available at FlyPush. Second, we have adopted an HA-tagged vector from the UAS Orfeome project to generate stocks with fly cDNAs inserted in attP sites on II and III for many protein coding genes. Third, we employ the same vector and insertion sites to create a small but growing number of human cDNA strains for the two closest human homologs of each conserved 4th protein coding gene. We have shown that fly and human UAS lines are functional by rescuing homozygous lethal T2A.GAL4 insertions in the cognate locus. Fourth, we deposited the mitotic (FRT101F) and meiotic (Blm) stocks reported in Goldsmith et al. (2022). Fifth, we are vetting the first six FRT101F chromosomes with a sequenced CRISPR mutation in a protein coding gene for unmarked and MARCM loss of function clones. Examples of strains in all collections will be shown. Inquiries and requests are welcome.

1045T **Exploiting single-cell RNA sequencing data in FlyBase** Damien Goutte-Gattat[1], Nancy George[2], Irene Papatheodorou[2], Nick Brown[11]Department of Physiology, Development and Neuroscience, University of Cambridge, [2]Gene Expression Team, European Bioinformatics Institute

Single-cell RNA sequencing has proved an invaluable tool in biomedical research. The ability to survey the transcriptome of individual cells offers many opportunities and has already paved the way to many discoveries in both basic and clinical research. For the fruit fly alone, nearly a hundred of single-cell RNA sequencing datasets have already been published since the first reported use of the technique in fly laboratories in 2017, a number that is only expected to grow quickly in the coming years. This increasing amount of single-cell transcriptomic data available, including whole- organism single-cell transcriptomic atlases, creates a challenge for biological databases to integrate these data and make them easily accessible to their users.

FlyBase is the Model Organism Database (MOD) for all data related to Drosophila melanogaster. It provides access to a wide range of scientific information either manually curated from the published literature or from high-throughput research projects. For single-cell RNA sequencing data, we aim to help fly researchers to: (i) discover the available Drosophila datasets; (ii) learn the most important informations about a dataset of interest; and (iii) get a quick overview of the expression data from those datasets.

To that end, we have set up a collaboration with the Single Cell Expression Atlas (SCEA), the EMBL-EBI resource for gene expression at the single cell level. FlyBase curators assist the EMBL- EBI's data scientists in obtaining and annotating Drosophila single-cell RNA sequencing datasets; in return, the SCEA provides FlyBase with the processed data in a standardized format, allowing for easier ingestion into our database. We then exploit the ingested data to enrich our gene report pages with specific displays for single cell expression data, giving our users an immediate view of the cell types in which a given has been found to be expressed.

1046T ***Drosophila Genomics Resource Center: research and reagent updates*** Daniel Mariyappa, Arthur Luhur, Kris Klueg, Andrew ZelhofBiology, Indiana University

Drosophila Genomics Resource Center (DGRC) is a repository for *Drosophila* cell lines and DNA reagents. Over the past three years over 100 DNA and cell culture reagents have been added to the DGRC catalog. These include several CRISPR/Cas9 vectors, gRNA collections, nanobody reagents, genome editing tools, attP-containing cell lines, tagged and knockout cell lines, and multiple Ras lines. Recently, a few mosquito cell lines and DNA reagents have also been donated. We will provide an update on the latest such reagents available at DGRC.

There are several ongoing research projects at DGRC, including the generation of recombination mediated cassette exchange (RMCE) compatible attP cell lines, generation of other novel cell lines, continued authentication of *Drosophila* cell lines and developing novel culture conditions. For instance, the attP sites in the RMCE-compatible lines were introduced at the same genomic sites as those in whole flies, thus providing the opportunity to compare data with *in vivo*

experiments. These lines have proved to be very popular with the DGRC having distributed several of the attP lines to multiple laboratories in the US and internationally. We continue to expand and validate the RMCE-compatible *Drosophila* cell lines, and generate novel cell culture reagents using these lines. We will provide an update on the ongoing DGRC research projects.

1047T **Optimization of cardiac optogenetic control for ChRmine opsin in *Drosophila melanogaster*** Fei Wang, Elena Gracheva, Abigail Matt, Hongwu Liang, Mathew Fishman, Chao ZhouWashington University in St. louis

Drosophila melanogaster has been widely used for cardiovascular studies with the benefits of a short life cycle, allowing for rapid testing for opsins for optogenetic purposes. Recently, newly-discovered ChRmine has aroused researchers' interest for its extreme light sensitivity, large photocurrent, and red-shifted spectrum. The combination of the features makes it promising for addressing one bottleneck of light delivery through tissue for non-invasive optogenetic pacing. Application in controlling mouse behavior through ~7 mm transcranial illumination has been demonstrated, but there was not much cardiovascular research using ChRmine. This study aimed to screen for the practical parameters for operating ChRmine in cardiac optogenetics. Pupa hearts were monitored by customed Optical Coherence Tomography with a synchronized LED module for pulsed illumination noninvasively in real-time. Data were processed using the lab-built software FlyNet2.0 for heart function quantification. The opsin expression level was controlled by incorporating different heart-specific drivers. Different illumination protocol (power density, pulse width, wavelength) was applied. *All-trans-retinal* was tuned by the concentration in the fly food. The frequency threshold for heart pacing of the animal was explored. The preliminary results verified the feasibility to pace the heart with ChRmine opsin and suggested that a higher expression level of the opsin can enhance the performance using low power.

1048T **Graphene enables optical control of Drosophila heart function** Abby Matt[1], Hongwu Liang[1], Matthew Fishman[2], Elena Gracheva[1], Fei Wang[1], Xinyuan Zhang[1], Alex Savtchenko[3], Chao Zhou[11]Biomedical Engineering, Washington University in St. Louis, [2]Computer Science, Washington University in St. Louis, [3]University of California San Diego

The field of noninvasive cardiac control has stemmed from a need to overcome challenges that come from traditional electric implantable pacemakers, which can require surgery, cause infection and damage to vessels, and require battery replacements. *Drosophila* have emerged as a powerful tool to study optical cardiac stimulation methods due to their ease of genetic manipulation, low-light scattering cuticle, and short lifespan. In this study, we show how reduced graphene oxide (rGO) can be used to increase the heart rate of yellow-white *Drosophila* pupa using light stimulation from 470 nm (blue light), 617 nm (red light), and 656 nm (near-infrared light). rGO is a monolayer, honeycomb arrangement of carbon molecules that generates electrons in response to visible light (400-700 nm). When injected into the heart tube of pupa, it aggregates in the heart tube and changes the membrane potential when light is shown, which leads to an increase in heart rate. Our study characterized the heart response to rGO stimulation, utilizing rGO's broad absorption spectrum. Optical coherence microscopy (OCM) was used to image a cross-section of the heart tube over time, providing information about heart rate, rhythmicity, and morphology. We found that the heart rate increase is dependent upon the power density level, showing a significant increase over the resting heart rate, an average of 20% higher than the resting heart rate at the maximum power density used, 6 mW/mm^2 in n=15 pupa. In addition, the heart rate showed an increase in response to all wavelengths of light used. This work is promising for use in both heart disease models, as rGO stimulation does not require prior genetic manipulation, and in larger animal models, where near-infrared light can be used to penetrate the heart when it lies deeper into tissue.

1049T **Orange maker: make red to orange.** Hee Su Park, Anna Gross, Seung-Jae Oh, Nam Chul KimUniversity of Minnesota Duluth

One of the greatest strengths of Drosophila genetics is its easily observable and selectable phenotypic markers. Mini-white has been widely used for *Drosophila* transgenesis as a transgenic marker. Flies carrying a mini-white construct can exhibit various eye colors ranging from pale orange to intense red, depending on the insertion site. Because the two copies of mini-white gene show a more stronger orange color, this is often used for selecting progenies after chromosomal recombination which causes the chromosome to contain two transgenes. However, some GAL4 lines available in the fly community originally have very strong red eyes. Without employing another marker such as GFP, it may not possible to generate a recombinant chromosome having the strong red eyed GAL4 and a desired UAS-transgene construct. Therefore, we decided to make red to orange using GAL4 lines having strong red eyes. To change the eyes color of the fly, we tested the CRISPR/Cas9 method with a guide RNA targeting the white gene against OK371-GAL4 and

Elav-GAL4. After a simple screening, we have successfully obtained multiple lines of orange eyed OK371-GAL4 and Elav-GAL4. Currently, we are verifying whether their GAL4 activity is affected during this process.

1050T **Resources for genome-wide mosaic analysis in Drosophila by MAGIC** Rhiannon Clements, Yifan Shen, Ann T Yeung, Bei Wang, Parker A Jarman, Chun HanDepartment of Molecular Biology and Genetics, Cornell University Weill Institute for Cell and Molecular Biology

The ability to generate mosaic animals allows for broad study of tissue-specific biological processes and precise single-cell analysis in vivo. The current mosaic tools in Drosophila commonly involve Flippase (Flp)/FRT recombination, limiting the ability to generate mosaic clones in fly lines lacking FRT sites. We have previously developed a novel mosaic technique called Mosaic Analysis by gRNA-Induced Crossing-over (MAGIC), which utilizes CRISPR/Cas9 to induce homology-directed repair (HDR) and chromosomal crossing-over at a predetermined target site(s), thus avoiding the need for a FRT site. To make the MAGIC technique accessible for genome-wide mosaic analysis in Drosophila, we aim to develop a complete toolkit that consists of three components: (a) an optimized MAGIC gRNA-marker collection for all chromosomal arms; (b) a collection of fly strains that express Cas9 in precursor cells of distinct tissues for accessible MAGIC applications; and (c) anti-CRISPR tools for limiting and fine-tuning Cas9 activity. We have used the MAGIC tools we already established to screen deficiency libraries for genes that are important for morphogenesis of larval sensory neurons and epidermal cells. Together, the reagents developed in this project will benefit the Drosophila community by providing versatile and powerful tools for genome-wide and tissue-specific mosaic analysis.

1051T **Utilizing the Drosophila Activity Monitors (DAM2) from TriKinetics to automate heat tolerance assays in *Drosophila melanogaster*** Blase Rokusek[1], Sunayn Cheku[1], Matthew Rokusek[2], Kimberly A. Carlson[11]Biology, University of Nebraska at Kearney, [2]School of Computing, University of Nebraska-Lincoln

The study of heat tolerance in *Drosophila melanogaster* is of particular interest to researchers since the discovery of the heat shock response. A common approach to assess heat tolerance in *Drosophila* is to monitor the time to knockdown after exposure to an elevated temperature. Generally, flies are housed in individual vials and placed inside a heated water bath. Time to knockdown or paralysis is then monitored manually by researchers. While very well-established, there remains an inherent amount of subjectivity to these assays. Recently, in the *Journal of Insect Physiology,* MacLeon et al. (2022) in the Jesper Sørensen lab described a reliable automated method for assessing heat tolerance using a video-based assay. In our lab, we have developed a similar high-throughput method for automating heat tolerance assays using the *Drosophila* Activity Monitors (DAM2) from TriKinetics. To accompany the DAM2 system, we have written a Python script to automatically read the last time of movement from the activity data generated. This script then writes to a csv file the time to heat paralysis for each fly. Our data shows that this method reliably differentiates heat hardened and control flies. Meanwhile, activity profiles created from the activity data themselves are of interest. These activity profiles show obvious group differences, with a notable difference being that the hardened flies show less overall activity during the heat stress event then do the control flies. This trend is consistent for both males and females. As global temperatures continue to rise, research involving heat tolerance and the ability of fruit flies and other insects to adapt to increasing temperatures will become increasingly relevant. Objective, high throughput automated alternatives to traditional observation-based heat tolerance assays could prove useful, especially when considering the novelty of activity profiles spanning the duration of the heat stress event prior to the time of paralysis. These types of data could allow for the investigation of previously unexplored aspects of thermal tolerance and stress-induced behavior in *Drosophila,* which could harbor relevant information and lead to a more complete understanding of heat tolerance in *Drosophila* as a process rather than an isolated moment at the time of paralysis. The project described was supported by grants from the National Institute for General Medical Science (GM103427 & 1U54GM115458).

1052T **Targeted protein degradation using nanobodies and the STUB1 E3 ligase domain in *Drosophila*** Ah-Ram Kim[1], Jun Xu[1,2], Norbert Perrimon[11]Department of Genetics, Harvard Medical School, [2]Key Laboratory of Insect Developmental and Evolutionary Biology, Chinese Academy of Sciences

RNAi and CRISPR are commonly used for loss-of-function studies. However, these approaches may not be well suited in the case of proteins with slow protein turnover rates, an issue that can be overcome with tools that induce protein degradation. In one approach, GFP fusion proteins can be degraded following ubiquitination using a nanobody against GFP tagged with an E3 ligase. To date, only one E3 ligase system, based on the Slmb E3 ligase domain (nSlmb), has been reported in Drosophila (Caussinus et al., 2011). To increase the repertoire and compare the efficiency of E3-ligase based degradation systems, we tested eight E3 different ligases (Ago, Ohgt, Rdx, Skp2, Slmb, Socs44A, STUB1, and Vhl) in

Drosophila cell lines. Specifically, we expressed anti-GFP nanobody tagged with the various E3 ligase domains in S2 and S2R+ cells and checked whether GFP-tagged proteins could be depleted. Among the E3 ligases, including the previously reported nSlmb E3 ligase domain, only the STUB1's E3 ligase domain was able to effectively degrade target proteins in *Drosophila* cell lines. Next, we tested the ability of fusions of the STUB1 E3 ligase domain to epitope-recognizing NanoTag nanobodies (NbVHH05 and Nb127D01, Xu et al., 2022) to target proteins tagged with the corresponding NanoTag epitopes. These additional nanobodies with STUB1 E3 ligase domain showed effective degradation of VHH05- and 127D01-tagged proteins, indicating the general versatility of this approach. The nanobody-STUB1 complex enabled degradation of nuclear, cytoplasmic, cell membrane-bound, and outer mitochondrial membrane-bound proteins. In addition, protein degradation using the nanobody-STUB1 approach reduced *in vivo* protein levels faster than RNAi-based methods, and without obvious toxicity when overexpressed. These results indicate that in combination with nanobodies, the STUB1 E3 ligase domain can act as a versatile protein degradation tool for *Drosophila* cell-based and *in vivo* studies.

1053F **Mosaic analysis by gRNA-induced crossing-over mediated by nickase Cas9** Ann T Yeung, Yifan Shen, Rhiannon Clements, Bei Wang, Chun HanDepartment of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University

Mosaic analysis is a powerful approach for studying tissue-specific gene function and cell-cell interaction *in vivo*. While most currently available mosaic techniques in Drosophila rely on the FRT/Flippase site-specific recombination system, we have recently developed a new method called mosaic analysis by gRNA-induced crossing-over (MAGIC). MAGIC takes advantage of the CRISPR/Cas9 system to generate double-stranded DNA breaks at defined target sites, which can induce chromosomal crossing-over through homology-directed repair (HDR) and subsequent mitotic recombination. While developing and using MAGIC, we noticed some limitations associated with the use of Cas9. First, double strand breaks (DSB) generated by Cas9 create a high frequency of non-homologous end-joining events (NHEJ) that mutate the target sequence, preventing subsequent cutting from occurring. Thus, early Cas9 action before the desired cell cycle phase or developmental stage can negatively impact clone induction. Second, NHEJ has a tendency to cause chromosomal aberrations, resulting in abnormal clones and cell ablation. To solve these problems, we explore the use of Cas9-derived mutant nickases, which have been successfully used for precise genome editing. In contrast to Cas9, nickases create single strand cuts (nicks) that are precisely repaired to the original sequence, which is then available for continuous nicking. Using our existing gRNA-marker transgenes, we found that nickases are able to induce more frequent clones than Cas9. 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. The results from this study will provide important insights for us to further optimize the MAGIC technique for safer and more efficient mosaic analysis.

1054F **Characterization of shock wave effects using fluorescent nanoparticles in syncytial embryos of *Drosophila melanogaster*** Daniel Tapia Merino, Juan Rafael Riesgo Escovar, Achim Max Loske Mehling, Pedro Salas CastilloUniversidad Nacional Autonoma de Mexico

In this work, fluorescent 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 perforate other cell membranes, such as those of fungal and bacterial cells. We used fluorescent carbon quantum dots synthetized by the hydrothermal method, and characterized them using UV-visible spectroscopy, fluorescence spectroscopy, and transmission electron microscopy. In our shock wave protocol, the majority of embryos treated with shock waves in a suspension of nanoparticles survived to adulthood. Centrifugation tests and histological sections suggest the presence of nanoparticles inside the treated flies. 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 shock waves, will open the door to diverse biological applications.

1055F **Development of hybrid RNA FISH protocol in *Drosophila* polytenes** Hannah E Gilbonio, Gwyn Puckett, Leila E RiederBiology, Emory University

Investigating interactions between proteins and DNA often requires determining whether a protein is binding to a specific locus. This can be visualized through combining DNA fluorescent in situ hybridization (FISH) with protein immunofluorescence (IF). However, DNA FISH requires a heating step that can reverse chemical crosslinks and denature protein epitopes making it a challenge to combine with fluorescent antibody staining of proteins. Furthermore, combining DNA FISH with IF is not very accessible for less-experienced trainees such as undergraduate researchers. To

address this technical issue, we developed an alternative strategy to be applied to *Drosophila* polytene chromosomes. Our solution to determine protein localization is to combine and optimize RNA FISH, which does not require a denaturation step, with protein IF. We hypothesize that RNA transcripts concentrate around their source gene providing an alternative source to obtain genetic location information. The objectives of this project are threefold: 1) Test the ability of RNA FISH probes to mark genomic loci on polytene chromosomes; 2) Determine if single molecule (sm)RNA probes are hybridizing to local RNA or DNA; 3) Combine and optimize RNA FISH with immunofluorescence. Ultimately, we aim to visualize proteins binding to single gene copy in the *Drosophila* genome. The histone locus (HL) provides an excellent model for our investigation. In the wildtype *Drosophila melanogaster* genome, there is a single locus consisting of five histone genes tandemly repeated 107 times. We can engineer fly lines to include a varying number of transgenic histone copies where even a single copy can attract transcription factors. We utilize *histone2b* RNA FISH probes and verify expected protein binding by immunostaining polytenes with Mxc, a specific marker of histone gene arrays. We have successfully visualized colocalization of *histone2b* FISH and Mxc IF at 107 and 12 histone gene copies, yet further optimization is required for us to resolve colocalization at a single histone gene copy. We hope to refine this technique so that it can be applicable for those working with *Drosophila* transgenes marked by reporters by leveraging smRNA probes targeting genes like *miniwhite*. Overall, our goal is to engineer a broadly applicable protocol for investigating and visualizing protein binding at a specific genomic location that is accessible to *Drosophila* investigators at all levels.

1056F **The Gene Disruption Project Update: An expanded toolkit for gene tagging using synthesized homology donor constructs for CRISPR mediated homologous recombination** Oguz Kanca[1], Jonathan Zirin[2], Yanhui Hu[2], Burak Tepe[1], Debdeep Dutta[1], Wen-wen Lin[1], Liwen Ma[1], Ming Ge[1], Zhongyuan Zuo[1], Lu-Ping Liu[3], Robert W. Levis[4], Norbert Perrimon[5,6], Hugo J. Bellen[1,71]M & H Genetics, Baylor College of Medicine, [2]Department of Genetics, Harvard Medical School, [3]Department of Genetics, Department of Genetics, [4]Department of Embryology, Carnegie Institution for Science, [5]Department of Genetics, [6]Howard Hughes Medical Institute, Harvard Medical School, [7]Department of Neuroscience, Baylor College of Medicine

Gene traps and protein traps are valuable genetic tools to study gene function. Most of these are artificial exons, which must be inserted in an intron between two coding exons to function as a gene or protein trap. However, about 50% of the Drosophila genes do not have a suitable coding intron for for this approach. We therefore developed a strategy to replace the coding region of these genes with a Kozak-GAL4-3XP3GFP cassette, creating a null allele, while expressing GAL4. We have generated >400 Kozak-GAL4 alleles to date and found that the mRNA expression pattern of Gal4 largely overlaps with the mRNA expression pattern of the targeted gene in the tested cases. Like T2A-GAL4 gene traps, the Kozak-GAL4 lines can be used to detect expression pattern, determine the mutant phenotype of the target gene and conduct rescue experiments through expression of a UAS-cDNA. To facilitate the generation of homology donor contructs for CRISPR-based insertion of T2A-GAL4 and Kozak-GAL4, we also generated new vectors. These circular donor vectors are linearized in vivo, improving the knock-in efficiency of large constructs (>5kb) flanked by short homology arms (200 bps). The short homology arms can be commercially synthesized to generate intermediate vectors, minimizing the cloning steps in donor generation. Our newest vector backbones include both gene specific sgRNA(s) and an sgRNA to linearize the donor, allowing co-delivery of all the components for targeting a gene in a single plasmid while increasing the successful transgenesis rate to 80%. The synthesized homology donor intermediate vectors can also be used to generate different homology donor constructs to modify the targeted gene with GFP protein traps or replace the gene with orthologous human cDNA. In summary, these upgrades, together with the Kozak-GAL4 strategy, will enable efficient targeting of 80% of the conserved fly genes by the Gene Disruption Project to generate gene and protein trap alleles.

1057F **Exploring Affects Within Drosophila's Central Complex Using Computational Affective Neuroscience** Saul GarnellComputer Science, Auckland University of Technology

My paper sheds light on general artificial intelligence by modelling a neuronal circuit of the seek affect within the central complex of *Drosophila melanogaster*. The paper hypothesizes that the 'seek affect' (one of seven known affects) are the root of phenomenal consciousness, and that by employing three domains of methodologies (mathamatics, neurophysiology, and computational affective neuroscience) it is possible to localize and model a neuromechanistic explanations of seek. The seeking system is the system that the proposal will focus on because, as Panksepp proposes, it makes up a foundational layer of consciousness apart from other affects. Animal models such as *Drosophila* are well suited for exploring affective circuits for several reasons. Firstly, the brain of Drosophila contains fewer neurons than most other animal models. For example, the fruit fly has roughly two hundred and fifty thousand neurons compared to humans, which have billions. Secondly, numerous well-established research methodologies can be leveraged since many researchers have heavily studied *Drosophila*. Finally, software platforms such as the Fruit Fly Brain Observatory (FFBO)

allow for (relatively) more straightforward ways to explore both the connectome and synaptome of *Drosophila*. The importance of this research is, central to man's understanding of a longstanding enigma: What is the cause of a living creature's subjective experience in the world? If *Drosophila* models help provide objective results that shed light upon the true nature of affective states, and by association, phenomenal consciousness, it would be a significant first step towards a comprehensive explanation of phenomenal consciousness and artificial general intelligence.

1058F **Imaging the Molecular Kinetics of Functional Nuclear Organization During Development** Apratim Mukherjee, Mustafa MirChildren's Hospital of Philadelphia

The proper regulation of transcription in space and time is critical for directing cell fate decisions during embryonic development. At the molecular scale, gene regulation involves dynamic protein-protein and protein-chromatin interactions at the scale of milliseconds to seconds. These interactions have been, until recently, impossible to quantify directly in their native context due to signal-to-background limitations imposed by traditional microscopy techniques. The advent of high-resolution lattice light-sheet microscopy has enabled the quantification of molecular scale kinetics of transcription factors in the context of transcriptional activity at their target genes in live, developing embryos. Here, I will show how recent advances in lattice light-sheet microscopy enable accurate single-molecule localizations and tracking, rapid volumetric scans and correlative multicolor imaging to study how emerging patterns of sub-nuclear compartmentalization during development modulate the kinetics of transcription factors and key components of the transcriptional machinery. I will show data on the application of this approach to understand how the formation of apical heterochromatin domains in the nuclei of blastoderm stage Drosophila embryos modulates the single molecule kinetics of RNA polymerase II. The imaging and quantification techniques introduced here will enable us to unveil how the molecular scale dynamics of transcriptional regulation is differentially modulated in transcriptionally silent versus transcriptionally active nuclear microenvironments during development in live embryos.

1059F **Quantification of properties of Zelda in *Drosophila* embryo using Raster Image Correlation Spectroscopy** Sadia Siddika Dima, Gregory ReevesTexas A&M University

The zygotic genome in most metazoans remains transcriptionally silent after fertilization and early embryonic development is controlled by maternally contributed mRNAs and proteins. 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. It binds to the nucleosomal DNA and increases chromatin accessibility thus facilitating the binding of other transcription factors (TFs) and early gene expression. The absence of Zld leads to embryonic lethality. However, the mechanism of nucleosome depletion induced by Zld is still unknown. Quantitative measurements of the biophysical parameters such as the nuclear concentration of Zld 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. Therefore, we performed Raster Image Correlation Spectroscopy (RICS) analysis on images of live embryos at nuclear cycle 11-14 carrying superfolder GFP-tagged Zld and H2A-RFP marker to extract information about molecular dynamics and concentrations. Analysis of the statistics of the autocorrelation function provides the quantification of the mentioned parameters over time. Incorporation of the results of the quantitative measurements in the models proposed earlier is expected to improve our understanding of Zld's role in TF binding and enhancer activation.

1060F **The *Drosophila* eye as a model for nanoparticle-based drug delivery.** Emily Brown[1], Tenley Spataro[2], Cristina Rodriguez-Quijada[2], Kimberly Hamad-Schifferli[3], Jens Rister[21]Biology, University of Massachusetts Boston, [2]University of Massachusetts Boston, [3]Engineering, University of Massachusetts Boston

Age-related macular degeneration (AMD) and diabetic retinopathy are the leading causes of vision loss in the United States. Both retinal diseases cause progressive degeneration of the posterior eye and require the delivery of therapeutics to slow progression. The most common method of therapeutic delivery is intravitreal injections. However, injections must pass through several biological barriers of the eye and pose a serious risk of inflammation or infection. Since the prevalence of AMD and diabetic retinopathy is steadily increasing, there is a critical need to develop alternative methods of therapeutic delivery. Here, we establish a nanoparticle-based drug delivery system in the *Drosophila melanogaster* eye to develop an alternative strategy to treat eye diseases. In our model system, our goal is to restore vision in blind *histidine decarboxylase* mutants that lack the enzyme required to synthesize histamine, the neurotransmitter of fly photoreceptors. Since, like in humans, the vitamin-A precursor β-carotene is

absorbed in the gut and targeted to the eye for the formation of retinal chromophore as well as the visual pigments, we use β-carotene and its derivative retinal as targeting molecules to direct histamine-carrying nanoparticles to the eye. To this end, we synthesize star-shaped gold nanoparticles that carry the targeting molecule β-carotene/retinal and the drug histamine to restore vision in blind *histidine decarboxylase* mutants. To control release, the nanoparticles are engineered to absorb near-infrared wavelengths which enter the *Drosophila* eye and act as a photo-trigger to induce the degradation of the gold nanoparticles. Successful targeting and release as well as premature drug leakage is determined by monitoring the behavior of *histidine decarboxylase* mutants in response to visual stimuli. Additionally, restoration of phototransduction, which is disrupted in *histidine decarboxylase* mutants, will be verified by electroretinogram. To determine if gold nanoparticles are successfully targeting the eye, we will use ICP-MS to identify tissues with large deposits of gold nanoparticles. Taken together, we are establishing a nanoparticle-based drug delivery mechanism in the *Drosophila melanogaster* eye that may inspire novel therapies to treat ocular diseases.

1061F **Dual-color optogenetic tool enables non-invasive heart pacing and restorable heart arrest in *D. melanogaster*** Jiantao Zhu[1], Elena Gracheva[1], Yuxuan Wang[1], Fei Wang[1], Matthew Fishman[1,2], Abigail Matt[1], Hongwu Liang[1], Chao Zhou[11]Dept. of Biomedical Engineering, Washington University in St. Louis, [2]Dept. of Computer Science and Engineering, Washington University in St. Louis

We have developed a new *D. melanogaster* optogenetic pacing system based on a transgenic line containing two opsins, ChR2 and NpHR2.0, and customized hardware involving optical coherence microscopy (OCM) imaging and a dual-color LED light pulse generation module. ChR2 is a member of the channelrhodopsin family, responding to blue light (~480 nm) and causing cells depolarization. NpHR2.0 is a halorhodopsin with sensitivity to orange/red light (~590 nm), which induces cells hyperpolarization. We combined ChR2 and NpHR2.0 transgenes using genetic tools and confirmed the presence of both transgenic constructs on the molecular level. The heart-specific opsins expression pattern was achieved by driving the expression with Hand-GAL4. Having an optogenetic system with a functional tissue activator (ChR2) and inhibitor (NpHR2.0) responding to discrete light wavelengths allowed us to create a sophisticated cardiovascular research platform in *Drosophila*. The initial experiments demonstrated the feasibility of increasing the heart rate following the designed blue light pulses and inducing the restorable heart arrest caused by prolonged red-light illumination in the same animal. Reducing the heart rate using red light with square pulses was also demonstrated. The main advantage of our system is that the heart function control was achieved non-invasively. We have optimized the OCM system parameters, such as irradiance level, pulse width and illumination schedule, to minimize the detrimental effects on live animals to ensure longitudinal studies on *Drosophila* models of human diseases, such as Type 2 diabetes.

Keywords: Optogenetics, Optical Coherence Microscopy, *Drosophila*, heart pacing, ChR2, NpHR2.0.

1062F **Engineered tandem duplications of varying sizes using CRISPR and recombinases** David LoehlinBiology, Williams College

Tandem gene duplicates are important parts of eukaryotic genome structure, yet the phenotypic effects of new tandem duplications are not well-understood, in part owing to a lack of techniques to build and modify them. We introduce a method, Recombinase-Mediated Tandem Duplication (RMTD), to engineer specific tandem duplications *in vivo* using CRISPR and recombinases. We demonstrate construction of four different tandem duplications of the *Alcohol Dehydrogenase* (*Adh*) gene in Drosophila melanogaster, with duplicated block sizes ranging from 4.5 kb to 21.0 kb. These *Adh* duplications show 1.4- to 2.3-fold higher ADH enzyme activity than unduplicated single copies. This indicates that expression of different-sized tandem duplicates of the same gene may depend on (yet unknown) modifying factors. The recombinase-mediated tandem duplication approach demonstrated here is combinatoric by design, opening the door to systematic study of the relationship between gene expression and the structure of duplications.

1063S **ModelMatcher: An online resource to facilitate cross-disciplinary collaborations between scientists, clinicians and beyond** Shinya Yamamoto[1,21]Department of Molecular and Human Genetics, Baylor College of Medicine, [2]Jan and Dan Duncan Neurological Research Institute, Texas Children's Institute

Advancement of clinical genomic technologies such as whole-exome and whole-genome sequencing is leading to identification of novel genetic causes of human diseases. In order to understand the functional consequences of disease-associated genetic variants and to reveal the underlying pathogenic mechanisms, it is critical to facilitate interdisciplinary collaborations between clinicians and basic scientists who share interests in the same/orthologous genes. While a number of national and regional efforts, such as the Undiagnosed Diseases Network (UDN) in the USA and Rare Diseases

Models and Mechanisms Network (RDMM) in Canada, have been established to facilitate such collaborations, efforts to stimulate international collaborations on a large scale have been lagging behind. To fill this gap, we have been developing ModelMatcher (https://www.modelmatcher.net), a bioinformatic tool that allows scientists to identify clinical collaborators on a global scale. Using this web browser based tool, which is connected to the MARRVEL search tool (http://marrvel.org/), RDMM International scientist registries (https://rdmminternational.org/) and MatchMaker Exchange clinical databases (https://www.matchmakerexchange.org/), *Drosophila* researchers can quickly gather information about the human and other model organism orthologs of their genes of interest and further identify potential clinical and scientific collaborators around the world. While there is enormous value in pursuing fundamental scientific questions that does not have obvious or immediate clinical value, active involvement in a clinical project may help further increase the significance of specific research projects, increasing the impact of the basic scientific work performed on the fly gene of interest.

1064S **A versatile automated robotic system for high-throughput microinjection of Drosophila embryos** Andrew D Alegria[1], Amey S Joshi[1], Jorge Blanco Mendana[2], Kanav Khosla[1], Kieran T Smith[3], Benjamin Auch[4], Margaret Donovan[4], John Bischof[5], Daryl M Gohl[6], Suhasa B Kodandaramaiah[71]Mechanical Engineering, University of Minnesota Twin-Cities, [2]University of Minnesota Genomics Center, University of Minnesota Twin-Cities, [3]University of Minnesota Twin Cities, [4]University of Minnesota Genomics Center, University of Minnesota Twin Cities, [5]Mechanical Engineering, Biomedical Engineering, University of Minnesota Twin Cities, [6]University of Minnesota Genomics Center, Genetics, Cell Biology, and Development, University of Minnesota Twin Cities, [7]Mechancial Engineering, Biomedical Engineering, Neuroscience, University of Minnesota Twin Cities

Microinjection is a widely used technique with broad applicability in basic research and clinical settings for transgenesis, mutagenesis, cell labeling, cryopreservation, and in-vitro fertilization. However, microinjection remains a highly specialized and labor-intensive procedure. Here we constructed a simple computer vision guided robot using off-the-shelf components to fully automate the process of microinjection in multiple model organisms. The robot uses multiple cameras to image embryos. Machine learning models are trained to detect individual embryos on agar plates and serially performs microinjection at specific locations in each detected embryo without any human intervention. We used three such robots operated by expert and naive users to automatically microinject Drosophila melanogaster (fruit fly) and Danio rerio (zebrafish) embryos. We systematically optimized robotic microinjection and validated the use of the robot for transgenesis and other applications in both species. We have shown that the robot can robustly perform transposon mediated transgenesis, PhiC31 integrase-mediated transgenesis, and CRISPR mutagenesis in Drosophila. The robot maintains comparable survival rates and can increase throughput by several-fold when compared to manual microinjection. We are using this automated microinjection system to carry out experiments requiring tens of thousands of injected embryos in both flies and zebrafish, including a CRISPR-based enhancer scanning mutagenesis screen across a large (27 kb) developmental enhancer region. We anticipate that this versatile automated microinjection system can be applied to carry out microinjections in a wide range of other organisms and can perform high-throughput microinjections to enable new types of microinjection-intensive experiments.

1065S **Expression of small tandem duplications of the Adh gene** Elise Root, David LoehlinWilliams College

Our lab has found that the expression of a tandem duplicated gene is often not twice the expression of the original gene. Possible explanations for this are that the distance between the two copies of the gene or the regulatory elements included in the duplication influence expression. We have made fly lines with duplications of various sections of the *Adh* gene region by inserting FRT sites into locations close to the gene on either side. These duplications are small or close to the gene, cutting off potential regulatory elements. Using these lines and ones with larger duplications, we hope to see which elements of a tandem duplication influence gene expression.

1066S **Fijiwings and FijiwingsPolarity: tools to measure tissue and cell size and polarity** Leonard DobensBBS, Univ Missouri, Kansas City

Fijiwings and FijiwingsPolarity are tools available for download at Sourceforge.com and may be used on any computer capable of running Java platforms including Mac OS X, Linux x86 or Microsoft Windows. The Fijiwings/FijiwingsPolarity packages are modified versions of the ImageJ software package that once set up and launched according to instructions offer a simple set of tools designed to interface with image files to process and analyze the size and trichome distribution in photomicrographs of Drosophila wings. The original Fijiwings uses a set of macros developed to conduct semi-automated morphophometric analysis of tissue size and cell size based on trichome density. The updated

FijiwingsPolarity used a set of macros to detecttrichome shape and polarity. Combined, these tools can automate the measure of wing size in pixels, cell size by detecting trichome density and cell polarity by detecting trichome shape. When combined with genetic tools to manipulate the activity of genes regulating these processes, changes various wing phenotypes can be detected and the semiautomated set of tools allows confirmation of known interactions and screens for new gene interactions. Over 4500 downloads of Fijiwings have been made and here we will present some changes in its original design and alert the community to its usefulness to teach students the power of its simple programming language to automate the process and analysis of objects within a microscope image.

1067S **Strong and heritable RNA knockdown using a self-cleaving ribozyme in *Drosophila*** Kevin G. Nyberg[1], Joseph Q. Nguyen[2], Eren A. Keles[2], Richard W. Carthew[1,31]Molecular Biosciences, Northwestern University, [2]Northwestern University, [3]NSF-Simons Center for Quantitative Biology

Although RNAi is a powerful RNA-knockdown tool, it is less effective at silencing RNAs that are localized in nuclei and other organelles. RNAi also only partially silences target gene expression. Here, we report powerful RNA knockdown in *Drosophila melanogaster* using a 111-nucleotide self-cleaving hammerhead ribozyme, a modification of a naturally-occurring ribozyme found in the parasite *Schistosoma mansoni*. The N79 ribozyme was inserted into four independent long noncoding RNA (lncRNA) genes using a scarless CRISPR/Cas9 HDR approach. Ribozyme-induced cleavage resulted in >95% destruction of 3' RNA fragments, as assayed by single-molecule RNA FISH and quantitative RT-PCR. smFISH results suggest that cleavage and destruction can even occur for nascent transcribing RNAs. To control for potential effects produced by the mere insertion of 111 nucleotides into the genes, a catalytically inactive ribozyme fragment was inserted into the same four lncRNA genes with little effect on the levels and intracellular distributions of the RNAs. RNA knockdown using self-cleaving ribozymes has potential applications for the study of not only lncRNAs but also other nuclear-localized RNAs, bifunctional RNAs, and specific splice-variants of protein-coding genes.

1068S **Generation and validation of pX-UASTattB for dose-dependent misexpression studies in Drosophila** Monika Singh, Jung Hwan KimBiology, University of Nevada, Reno

The GAL4-UAS binary gene expression system has benefited genetic studies tremendously. However, tools for effective control over the expression levels of transgenes are largely limited. We report a new series pUASTattB-based plasmids. These plasmids preserve the features of pUASTattB but contain a varying number of UAS sites. The expression levels and biological outcomes of a transgene showed a dosage-dependency with the number of UAS sites when using Dscam1 as a transgene and axon arborization of Drosophila sensory neurons as a biological function. Our new plasmids provide novel and useful tools for
Drosophila genetic studies.

1069S **pBS-GMR-eya2(shRNA), an improved vector for visible counter-selection against vector insertion during CRISPR gene editing** Nathan Heyen, Gregory J Beitel, Hans AubeeluckMolecular Biosciences, Northwestern University

We had previously developed the pBS-GMR-eya(shRNA) vector for visible counter-selection against vector insertion during CRISPR or other gene editing procedures (Nyberg *et al.*, Fly 2020; DGRC plasmid number 1518). The original pBS-GMR-eya(shRNA) vector consists of pBlueScript, with its standard multiple cloning site, and a GMR enhancer driving an shRNA against the *Eyes Absent* (*Eya*) gene that produces small-eyed progeny having a *Drop* (*Dr*)-like phenotype if a vector insertion event occurs. The advantages of the *Eya*(shRNA) marker over other counter-selection markers are that the Eya phenotype can be scored in visible light, with enough of the eye being left for color or fluorescence to be scored, and that the GMR-Eya(shRNA) cassette is only 1kb instead of 4 kb for mini-white (not actually that mini), which simplifies plasmid construction.

While the original eya(shRNA) vector works well, we discovered that the sequences flanking the EcoRV site, which is commonly used for Gibson cloning, is flanked by a highly GC-rich region on one side, and a region that behaves sub optimally as a PCR primer on the other side. These sequences hamper designing effective primers to insert fragments into the Eya vector by Gibson cloning. We are therefore inserting sequences that should be better behaved to flank the EcoRV site and thus improve the utility of the Eya vector for building repair templates. The results of these modifications will be presented.

1070S **Optimization of Cut&Run for transcription factors during Drosophila zygotic genome activation** Puttachai Ratchasanmuang[1], Alan Boka[1], Joseph Zinski[2], Mustafa A Mir[11]The Children's Hospital of Philadelphia, [2]Department of Cell and Developmental Biology, University of Pennsylvania

Differential gene expression directs cell fate determination during embryogenesis . Gene activity is regulated through the binding of transcription factors to specific target sites. Chromatin Immunoprecipitation Sequencing (ChIP-seq) and related methods are well-established to map genome wide transcription factor binding profiles. However, ChIP-seq has several practical limitations including large sample sizes, high background and biased peaks resulting from cross-linking. As an alternative, Cleavage Under Targets & Release using Nuclease (Cut&Run), has been shown to address these issues in Drosophila cells, post-gastrulation embryos and larval tissues. However, Cut&Run has not been successfully demonstrated for studying transcription factor binding in syncytial blastoderm stage embryos. Here, we present an optimized Cut&Run protocol that we use to characterize the binding of a pioneer transcription factor, Zelda, during zygotic genome activation embryos. With this modified Cut&Run protocol, we can now characterize the binding profile of transcription factors in early Drosophila embryos using smaller sample sizes and reducing artifacts resulting from sample fixation in ChIP-seq.

1071S **A fluorescent sex-sorting technique for insects with the demonstration in Drosophila melanogaster** Junru Liu, Danny Rayes, Omar AkbariBiological Science, University of California, San Diego

Recent advances in insect genetic engineering offer new solutions to population controls for pests and vectors, including the Sterile Insect Technique (SIT) for population limiting or the “release of insects with dominant lethality” (RIDL) system for population suppression. Most genetic control methods are achieved by only releasing male insects into the field. While success has been achieved in the laboratory for those techniques, sex-sorting has been a limiting factor for the field trial as it requires a significantly larger number of insects than in laboratory settings. However, the current sex-sorting method requires insects to be manually sorted based on their gendered morphological differences. This process is time-consuming, labor-intensive, and, most of all, error-prone. Here we describe the development of a novel sex-sorting technique that utilizes fluorescent protein markers and the female-specific intron of the sex-determination gene Transformer (Tra) with a proof-of-principle in D. melanogaster. In the sex-sorting gene cassette, both genders express eGFP fluorescence, while only females express dsRed fluorescence. The female-specific fluorescent expression is achieved by incorporating Transformer female-specific introns (traF) to disrupt the coding sequences of the fluorescent marker gene. Under the sex-specific alternative splicing mechanism, the female-specific introns are only properly spliced out in females, resulting in correct coding sequences of the fluorescent gene, thus expressing the dsRed fluorescent in a female-specific manner. In this project, traF from four different species were tested, including Drosophila melanogaster (D. mel), Drosophila suzukii (D. suzukii), Ceratitis capitata (C. capitata), and Anastrepha ludens (A. ludens). When traF from D. mel, D. suzukii, C. capitata is inserted into the coding sequence of dsRed, 100% of the female adults express dsRed. Notably, the cassette with C. capitata traF permits the selection of females as early as L2 instar stage. However, A. ludens traF resulted in dsRed expression in both genders in D. mel flies, indicating that A. ludens traF is not sex-specific when transcribed in D. mel. This technique would be a valuable method for insect sex-sorting as it (1) exploited the highly conserved sex-specific splicing mechanisms, making it widely transferable to different insect species; (2) allows 100% positive selection for either females or males based on fluorescence instead of morphological differences, providing better accuracy; (3) permit sex-sorting during different life stages and as early as L2 instar stage; (4) can be combined with existing genetic control methods to use for a Complex Object Parametric Analyzer and Sorter (COPAS) machine for precise high-throughput screening.

1072S **A computational approach for determining developmental gene expression trajectories and combinatorial gene regulatory patterns in fly motor neurons** Erdem Varol[1,2], Himanshu Pawankumar Gupta[1], David Stern[3], Peter A Sims[1], Richard Mann[11]Columbia University, [2]New York University, [3]Janelia Research Campus, HHMI

Decoding the gene regulatory networks that determine neural identity, function and connectivity is an ongoing challenge that has become rapidly bolstered by advances in high-throughput single-cell RNA sequencing. Although several breakthroughs have been made in high-resolution genomic profiling of various parts of the fly nervous system, such as the optic lobe and the mushroom body, there hasn’t yet been a comprehensive characterization of the transcriptional landscape of leg motor neurons. A complete temporal gene expression profiling of leg motor neurons (LMNs) has the potential to elucidate genetic programs that determine their function, namely their differential connectivity to leg muscles and, ultimately, fly locomotory behavior. Here we set out to develop a computational toolbox to help achieve this goal that can be applied to many other developmental systems.

We first introduce an approach to link neuron-type clusters across multiple time points that build on techniques from optimal transport theory. By using transcription factor (TF) expression in un-annotated neural clusters captured at four different time points (late L3, 20 hours after pupal formation (APF), 45 hours APF, and 1-day old adult flies), we match

corresponding clusters at sequential time points by optimizing a global objective function that minimizes the total number of TF edits between sequential time points. This approach gives us an unbiased way to link LMN clusters over time and then quantify the temporal gene expression patterns for all other genes.

Second, given temporal gene expression patterns for LMNs, we introduce another approach to predict and quantify the combinatorial TF code that regulates the downstream gene families for each LMN. Our approach differs from existing gene regulatory network analysis techniques in that we can efficiently capture the regulatory effect of higher order TF codes (dimer, trimer, etc.) on regulating a diverse set of gene families. Our results suggest a sparse set of TF combinations that may play a role in determining downstream phenotypic differences in LMNs due to differential gene regulation. Furthermore, ranking the TF codes by their statistical significance allows an efficient prioritization of ongoing gene knockout validation experiments.

Open source MATLAB toolbox is available upon request.

1073V **REDfly: The Regulatory Element Database for *Drosophila* and other insects** Soile V. E. Keränen[1], Xinbo Huang[2], Marc S Halfon[31]None, [2]Center for Computational Research, University at Buffalo-State University of New York, [3]NY State Center of Excellence in Bioinformatics & Life Sciences; 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 has as of early November 2022 increased to contain data on more than 58,000 tested or predicted regulatory sequences and about 2700 TFBSs or their variants. By including as many functionally tested or predicted sequences as possible, both with and without observable regulatory activity, we aim to maximize the utility of the knowledgebase for purposes ranging 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.

CRM data in REDfly are broadly segregated into three categories: reporter gene analysis, deletion of potential regulatory sequences from the genome, and predictions based on, e.g., ChIP-seq, ATAC-seq and/or computational modeling. Also, we use the CRM data internally to infer minimally active sequences from tested CRMs. Transcription factor binding sites are mapped to overlapping CRMs.

REDfly CRM activity is annotated using structured anatomy, staging, and biological process ontologies. The anatomy terms, which may come from different papers, are linked to their original PubMed references, allowing the user to further explore the source data. While ”CRM” is often used interchangeably with ”enhancer,” REDfly also curates silencers, which inhibit gene transcription, often while acting as enhancers for some other location or condition.

New in 2023 will be a customized JBrowse interface for visualization of all REDfly data in their genomic context. Updates to REDfly’s search and download features are under development and will be rolled out over the course of the coming year, and there are more CRMs and TFBSs in the pipeline. REDfly is freely accessible at http://redfly.ccr.buffalo.edu and can be followed on Twitter at @REDfly_database.

1074V **Drosophila: A metazoan model to study the biological functions of inorganic polyphosphate** Sunayana Sarkar[1], Harsha Sharma[1], Jayashree Ladke Suresh[2], Sreejith Rarankurussi[1], Rashna Bhandari[2], Manish Jaiswal[11]BIOLOGY, TATA INSTITUTE OF FUNDAMENTAL RESEARCH HYDERABAD INDIA, [2]Centre for DNA Finger Printing and Diagnostics

Inorganic polyphosphate (polyP), which consists of chains of orthophosphate residues, is found in all living organisms. The biological functions of polyP have been extensively studied in prokaryotes and unicellular eukaryotes, however, their functions in metazoans are largely underexplored. Recent advancements predict numerous functions of polyP due to its presence in various cell types and its specific binding with several proteins. However, the major limitation in testing the functions of polyP in metazoans is the elusivity of the genes involved in polyP synthesis and turnover, which restricts modulation of polyP levels in vivo. We have developed a Drosophila model to study the functions of polyP. Here we show that polyP exists in flies and that its levels are developmentally regulated during major life cycle stages. We also show the cellular distribution pattern of polyP. We have been able to stain polyP in fly tissues and also find a developmental regulation of polyP in oogenesis. We have further created transgenic fly lines to manipulate polyP to study its functions. Overall we have developed Drosophila as a first genetic model to study polyP functions in metazoans.

1075V **Live embryo confocal microscopy screen to capture protein localization signals** Sumaiya Hasan[1], Brandon Bernicky[2], Ben Lundy[2], Andrew Melaga[2], Shaniya Barrett[2], Katelyn Tenuto[2], Kevin Edwards[21]Biological Sciences, Illinois State University, [2]Biological Science, Illinois State University

The intracellular environment is highly structured, with myriad protein-protein (and -lipid) interactions controlling the subcellular location of each protein; localization in turn regulates the protein's interaction network, activity, and stability. To identify novel protein localization signals in an unbiased manner, we tested the effectiveness of screening fluorescent protein traps in live Drosophila embryos with laser scanning confocal microscopy. The traps were generated with the Hostile takeover (Hto) system, in which GAL4 drives expression of an mCherry fusion to a downstream coding region (either a full-length form or C-terminal fragment of the target protein). In a pilot screen, we recovered all embryos expressing a fusion protein with a pattern distinct from that of the Starter Hto element. Of 79 selected and hatched embryos, 66 eclosed as adults, 56 were crossed, 55 transmitted the fluorescent tag to offspring, and 46 yielded stocks with new stable protein trap insertions. Localization was assayed for all lines using egg chamber follicle cells. Most (63%) of the fusions were concentrated in the nucleus, with several subtypes (nucleolar, non-nucleolar, and patterned), while another 11% transited from the cytoplasm to the nucleus following expression in mid-oogensis. The remaining 26% were mostly non-nuclear, including several with distinct patterns (junctions, vesicles, and plasma membrane). Thus, live embryo Hto screening provides an efficient means of harvesting protein fragments with diverse and useful localization signals. We further used the recovered lines to test whether enhanced resolution microscopy (via deconvolution) could uncover novel features of localization patterns. In the range of ~100-300 nm, deconvolution succeeded in refining discrete patterns, even with low signal, and improved the accuracy of colocalization.

1076V **Development of Gal4/UAS system for elucidating the mechanism of the wing color pattern formation of *Drosophila guttifera*** Masato Koseki[1], Shigeyuki Koshikawa[1,21]Hokkaido University, Graduate School of Environmental Science, [2]Hokkaido University, Faculty of Environmental Earth Science

It is challenging to explore the relationship between gene function and morphological diversification in Evolutionary Developmental Biology (Evo-Devo). To identify gene expression necessary for the formation of various animal bodies, researchers need to develop suitable gene manipulation methods for gene functional analysis in each animal.

Various black pigmentation patterns are seen in adult wings of *Drosophila* fruit fly species and have been regarded as a model of morphological diversity. The pattern formation mechanism has been examined in some species, including a polka-dotted fruit fly, *Drosophila guttifera*. The expression of a developmental regulatory gene, *wingless*, induces the pattern formation in the mid-pupal stage in this species. To understand how the wing color pattern of *Drosophila guttifera* was gained in detail, it is important to identify genes involved in the color pattern formation other than *wingless*. However, it was difficult because a suitable gene manipulation method has not been developed in pupal wings of *Drosophila guttifera*.

In this study, we tried to develop the gene manipulation method, Gal4/UAS system, in *Drosophila guttifera* by making functional Gal4 driver lines. Each line was designed to express *Gal4* gene at the mid-pupal or late-pupal stage by the upstream enhancer sequence. First, we made the candidates of these Gal4 lines by germline transformation with the *piggyBac* transposon vectors. Next, we detected the degree of *Gal4* expression of the candidates by *in situ* hybridization and found that some of them strongly expressed *Gal4*, suggesting these lines expressing *Gal4* at the mid-pupal or late-pupal stage are functional and can be used for gene manipulation.

Finally, these lines were crossed with the UAS-GFP-Tubulin lines to confirm whether Gal4/UAS system properly works in their F_1 generation. As a result, the reporter fluorescence was observed in the F_1 individuals expressing *Gal4* at the late-pupal stage, but not in the F_1 individuals expressing *Gal4* at the mid-pupal stage. The inhibition of the reporter expression was probably caused by the inappropriate 3' UTR of *gfp-tubulin* gene or/and insertion site of the *UAS* alleles in their genome.

1077V **Automated quantification of cardiac parameters and aging prediction using machine learning in a Drosophila model** Yash Melkani[1,2,2], Aniket Pant[2,3], Girish Melkani[21]Engineering Physics,College of Engineering, University of California, Berkeley, [2]Department of Pathology, Division of Molecular and Cellular Pathology, Heersink School of Medicine, University of Alabama at Birmingham, [3]Materials Science and Engineering, Georgia Institute of Technology

The *Drosophila* model has proven tremendously powerful for understanding the pathophysiological basis of several disorders including aging, metabolism, and cardiovascular disease. Relevant high-speed imaging and high-throughput

lab assays generate large volumes of high-resolution videos, necessitating next-generation methods for rapid analysis. We evaluate the use of deep learning models for automatic segmentation of Drosophila cardiac optical recordings for *Drosophila* aging models. We have demonstrated that neural architectures can generate high-quality segmentations for *Drosophila* heart beating-patterns. A total of 114,750 images and 1,437,600 images are used for training and experimental validation, respectively. These segmentations are used to calculate cardiac physiological parameters for young and old flies. Calculated parameters include heartbeat frequency, cardiac performance, arrhythmia index, diastolic and systolic intervals, and diastolic and systolic diameters for modeling cardiac function during aging. By coupling these segmentations and statistical calculations, we verify the aging model and identify age-dependent deceleration of cardiac performance and increased dysrhythmia. We experimentally validated our model using canonical software for *Drosophila* cardiac video analysis. Using a machine learning and deep learning method, we demonstrate two pipelines for prediction of heart age with and without access to cardiac statistics, demonstrating excellent experimental accuracy. Our findings are significant because we demonstrate the first use of deep learning for the automated quantification of all cardiac parameters in the aging model. To our knowledge, this is the first platform for deep learning-assisted segmentation applied to standard high-resolution, high-speed optical microscopy of *Drosophila* hearts and is the first to quantify all relevant parameters, including directly quantifying ejection fraction and aging prediction. Furthermore, this is the first work to demonstrate that neural models can capture rhythmic patterns in *Drosophila* cardiac recordings for identifying aging phenotypes and predict fly age. This machine-learning approach can be used to expedite future cardiac assays for modeling several diseases in flies and can be extended to numerous animal/human cardiac data analyses under multiple conditions.

1078V **Improved CRISPR systems for the generation of highly penetrant loss-of-function phenotypes in *Drosophila***
Fillip Port, Roman M Doll, Martha A Buhmann, Florian Heigwer, Michael BoutrosGerman Cancer Research Center

CRISPR genome engineering has transformed the way scientists can modify the genome to probe for causal genotype-phenotype relationships. Over the past few years several large-scale sgRNA libraries have been developed for conditional loss-of-function mutagenesis in *Drosophila*. These consist of collections of transgenic strains expressing one or two sgRNAs under a ubiquitous promoter or the Gal4/UAS system. Mutagenesis with these resources is highly robust, but frequently generates genetic mosaics harboring cells with one or two functional gene copies. Mosaicism is typically caused by limited CRISPR activity or the induction of silent (e.g. in-frame) mutations and can attenuate phenotypic penetrance. Here, we present two strategies to improve CRISPR knock-out experiments. First, we introduce a system that allows for higher order sgRNA multiplexing, which results in frequent generation of larger deletions that are more likely to abrogate gene function. Using quantitative comparisons of CRISPR induced phenotypes across many targets we show that this system outcompetes current tools in the ability to reveal loss-of-function phenotypes. Second, we developed a system that allows for the efficient and selective induction of precise mutations, such as premature stop codons, resulting in highly penetrant phenotypes that often phenocopy heritable null alleles. Together these novel CRISPR systems constitute a significant improvement in our ability to knock-out genes in various tissues of *Drosophila*.

1079V **Tools for investigating the subcellular distribution of channels in motion-sensing neurons of *Drosophila***
Renee Vieira, Eleni Samara, Sandra Fendl, Alexander BorstMax Planck for Biological Intelligence

Voltage-gated ion channels are important neural circuit components that shape the electrical activity of the neuron. Importantly, the subcellular distribution of these channels determines how signals are integrated. Knowing the localizations and types of ion channels is therefore fundamental to our understanding of neural computation. Though the fruit fly *Drosophila melanogaster* has unparalleled genetic accessibility, tools for endogenously labelling proteins in a cell-type-specific manner were missing. To this avail, we developed FlpTag, a tool for endogenous, conditional labelling of proteins using a flippase-dependent, invertible GFP cassette integrated into the gene of interest. FlpTag is generalizable and can be easily integrated into genes containing ΦC31 attP sites such as those of the vast MiMIC library, or specifically targeted to a gene locus of interest using the CRISPR-Cas9 gene editing system. This permits the tagging of virtually any protein within the fly using FlpTag. To expand this system, we developed SingleFlp, a new addition to the FlpTag technique which permits cell type specificity with single cell resolution. SingleFlp uses a multi-recombinase approach to endogenously tag proteins in single cells of a genetically defined type. Using these methods, we investigated the subcellular distribution of ion channels in T4/T5 neurons. We discovered a strictly segregated subcellular distribution of voltage-gated ion channels within different compartments of T4/T5 neurons. These findings lay the foundation for future functional investigations of ion channels within these neuronal cell types. Additionally, these strategies can be expanded to different circuits or even different species in the future.

1080T **A CRISPR screen for 5' UTR mutants affecting *gurken* translation in the context of a sophomore genetics lab** Scott B. FergusonBiology, State University of NY at Fredonia

Using the Course-based Undergraduate Research Experience (CURE) model, I have incorporated my research on the translation regulation of *gurken* into a genetics lab at SUNY Fredonia that enrolls approximately 60 students each year. The Gurken morphogen directs axis specification during *Drosophila* oogenesis through the well-characterized signaling interactions between Grk in the oocyte and the EGFR in the overlying follicle cells. The *grk* mRNA is subject to significant regulation to ensure that it is not translated prior to its localization to the dorsal anterior of the oocyte during mid oogenesis. My lab has demonstrated that *grk* translation can be promoted by dietary restriction. In an effort to identify the *cis*-acting sequence in the 5' UTR that control this response, we have performed a CRISPR-mediated deletion screen. Working in pairs, each student group is provided with transgenic flies expressing pairs of sgRNAs that were designed and cloned in a different upper-level lab course. Through a four-generation crossing scheme, students isolate novel mutations and create double-balanced stocks in a sensitized background. This portion of the lab illustrates the Mendelian principles of segregation and independent assortment as students manage the genotype of their flies by following phenotypic markers. During the second half of the lab students are introduced to molecular biology techniques and screen their fly lines for InDels using PCR and capillary electrophoresis. Lines that contain InDels are then sequenced and students map their mutations through alignment to the wild type sequence. The lab culminates in an assessment of the phenotypic consequence of their mutations. The framework of the lab provides a framework to adapt it to a large number of students, yet the stochastic nature of NHEJ repair enables students to generate their own unique mutations which gives them a sense of investment and ownership of the project. The strategy implemented here addresses the learning goals for most genetics courses and is readily applicable to mutagenesis of any target or any number of students.

1081T **The Genomics Education Partnership: Accessible and Equitable Research Opportunities** Raffaella Diotti[1], Martin G. Burg[2], Paula Croonquist[3], Justin R. DiAngelo[4], Jennifer Jemc[5], Christopher J. Jones[6], Jennifer Kennell[7], Judith Leatherman[8], Wilson Leung[9], Hemlata Mistry[10], Alexis Nagengast[10], Chinmay P Rele[11], Katie M. Sandlin[11], Jamie Siders[12], Stephanie Toering Peters[13], Norma Velazquez-Ulloa[14], Jacqueline Wittke-Thompson[15], Laura K. Reed[111]Bronx Community College, [2]Grand Valley State University, [3]Anoka Ramsey Community College, [4]Penn State Berks, [5]Loyola University Chicago, [6]Moravian University, [7]Vassar College, [8]University of Northern Colorado, [9]Washington University in St. Louis, [10]Widener University, [11]Biological Sciences, University of Alabama, [12]Ohio Northern University, [13]Wartburg College, [14]Lewis and Clark College, [15]University of St. Francis

The Genomics Education Partnership (GEP; thegep.org) is a consortium of over 200 institutions ranging from community colleges (CCs) and Primarily Undergraduate Institutions to R1 universities, and includes Minority Serving Institutions of both Historically Black Colleges and Universities and Hispanic Serving Institutions. GEP aims to integrate Course-based Undergraduate Research Experiences (CUREs) centered in genomics and bioinformatics into the undergraduate curriculum with the goal of offering authentic research opportunities to diverse student populations. Active participation in the research is supported by the GEP web-based platform with curated curriculum that can easily be incorporated into existing courses. To increase research opportunities for underrepresented students, GEP has focused on recruiting new members from CCs to broaden participation and to provide CC students equitable access to research opportunities traditionally available to four-year students. Using only a computer, internet access, and the GEP curriculum, students learn to annotate protein-coding genes in eukaryotic genomes, leveraging sequence similarity to related informant species, experimental data (e.g., RNA-Seq), computational gene predictions, and basic molecular biology rules to create a defendable gene model. Research projects include investigations of venom evolution in parasitoid wasps, the evolution of insulin pathway genes across 32 *Drosophila* species, and F element expansion in four *Drosophila* species. Additional research projects covering other species are actively being explored. GEP offers centralized support for member institutions including professional development for faculty, access to virtual teaching assistants for students, and publication with student/faculty co-authors on project meta-analyses and of the individual gene models in *microPublication Biology*, with students as co-authors. The GEP also engages in science education research. Our recent studies suggest that student attitudes towards science impact student learning outcomes. Early findings also suggest that CC students show similar benefits from the GEP experience compared to non-CC students, which could help mitigate barriers for CCs to implementing CUREs. GEP faculty benefit from the support of a regional node network encompassing 13 geographic regions across the U.S. and Puerto Rico. Our community of practice is welcoming new members to increase the network's diversity; contact us at thegep.org/contact. Supported by NSF IUSE-1915544, NSF RUI-2114661, and NIH IPERT-R25GM130517.

1082F **An improved gene trapping lab for introductory cell and molecular biology courses**

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For several years we have offered a one-semester gene trapping practicum that is designed to accompany an introductory (200 level) Cell and Molecular Biology course. This exercise utilized the WeeP system in which a promoterless EGFP-containing exon is mobilized through P-element transposition to land in random introns, whereupon the EGFP exon becomes spiced into the pre-mRNA and tags the host gene's protein. Two crosses are done, the first to introduce the Δ2-3 transposase and the second to generate F2 offspring that are heterozygous for germline insertions in the F1 fathers.

Several steps have been optimized for the teaching laboratory including screening for visible fluorescence in F2 larvae obviating the need for in-class anaethesia, and the utilization of a simple filtration strategy to isolate the candidate larvae from the food. Using these methods, a section of 24 students can screen 250,000-500,000 larvae in one semester, providing ample numbers to detect several successful gene trapping events. Follow up exercises involve DNA extraction and inverse PCR followed by sequence analysis for identification of the trapped gene, and fluorescent microscopy to identify the expressing tissues and to make an initial evaluation of subcellular localization.

Here we highlight a recent retooling of this construct to make this exercise more classroom friendly. First, we have replaced EGFP with tdTomato to escape the significant autofluorescence at shorter wavelengths that makes bona fide gene trap identification harder for the students. tdTomato is also significantly brighter than EGFP. Second, we have optimized some features of the transposon itself: (i) We created designed (as opposed to opportunistic) iPCR primers and an optimally localized group of restriction sites to maximize the likelihood of successful iPCR; (ii) We introduced longer linkers between the host protein and the tdTomato to lessen disruption of the host protein and hopefully increase the likelihood of obtaining a successful trap. (iii) Since we are directly selecting for fluorescence in our screen we created our lines using AttB/P insertion, moving the P[w+] gene out of the P-transposon, obviating the need for its subsequent removal using FLPase. The poster will be accompanied by a detailed syllabus and protocols.

1083F **Melanogaster: Catch The Fly!: a citizen science network in adaptation genomics** Josefa Gonzalez[1], Miriam Merenciano[2], Sonia Casillas[3], Marta Coronado-Zamora[1], María Bogaerts-Márquez[4], Ewan Harney[1], Irene Gonzalez[5], Roberto Torres[51]CSIC, [2]UCBL-1, [3]UAB, [4]CBGP, [5]LCATM

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 a research project, thus providing them with a unique opportunity to learn about the scientific process, while they contribute to facilitate and expedite scientific research. MCTF! 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 investigate the effects of climate change on local biodiversity.

MCTF! Citizen Science project is co-lead by the outreach platform LCATM (https://lacienciaalteumon.cat/) and the Evolutionary and Functional Genomics laboratory (gonzalezlab.eu) (Institute of Evolutionary Biology, IBE, CSIC, UPF. Spain), in collaboration with FECYT-Ministerio de Ciencia e Innovación, ERC and FGCSIC.

1084S **Teaching inheritance of traits using a model organism: Implementing inquiry-based learning** Suparna Chatterjee, Surya Jyoti BanerjeeBiological Sciences, Arkansas Tech University

The objective of this study was to implement inquiry-based learning to actively engage students who are preservice teachers in a science education course for elementary teachers. Educators and researchers have identified the need for developing science teaching methods for K-12 schools in the United States. It is critical for stimulating students' interest and enhancing their problem-solving ability, higher-order thinking skills, and performance in science content areas. For this research, an activity was created for studying the phenomenon that animals have traits inherited from

parents. The phenomenon was chosen based on the performance expectations in the Next Generation Science Standards (3-LS3-1). The activity implemented the 5-E model of inquiry-based instruction which focuses on the five phases such as engagement, exploration, explanation, elaboration, and evaluation. *Drosophila,* the fruit fly, was used as a model organism for studying the phenomenon. There are several contrasting traits available in different fly strains in the natural population such as red versus white eye color and straight versus curly wing which are useful to demonstrate patterns of inheritance in the animal world. Preservice teachers who are students in the science methods course completed the activity by analyzing and interpreting the phenomenon of inheritance of traits in *Drosophila*. Students developed a lesson plan using the 5-E model of inquiry instruction for teaching the phenomenon to elementary grade level students and presented a poster in the class. Data source for this study consists of open-ended surveys students completed after completing the activity, lesson plan, and poster presentation. Data were analyzed qualitatively using the thematic analysis method. Results showed i) a high level of engagement, interaction, and participation in students due to the hands-on experience in understanding the phenomenon, ii) students' comfort and confidence in facilitating the content, i.e., inheritance of traits from parents, iii) practicing inquiry-based learning and using it for developing lesson plans, and iv) modeling activity for students who are future teachers for their own classroom.

1085S **LacApp: A platform to help students master gene regulation through retrieval practice** Tom Torello, Paul Wolujewicz, Caitlin HanlonBiology, Quinnipiac University

In *Vision and Change in Undergraduate Biology Education: A Call to Action*, the American Association for the Advancement of Science identified gene regulation as a core biological concept (AAAS, 2011). A simple model for how gene expression is regulated is the bacterial lactose operon, which consists of a cluster of genes involved in metabolism of the sugar lactose, the DNA sequences that regulate the expression of those genes, and the regulatory proteins that bind to those DNA sequences. 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. To test this hypothesis, we have developed a tutorial called LacApp which 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. Here, we describe the design and pilot testing of LacApp with quantitative and qualitative data. Additionally, we are hoping to expand the testing and usage of LacApp, so if you are interested in using this tool in your undergraduate genetics course, please reach out!

1086T Tissue-specific mutagenesis of spastin, mediated by CRISPR/Cas9, to elucidate neuronal or glial function at the NMJ Emily Ozdowski Duke University

Autosomal Dominant Hereditary Spastic Paraplegia (AD-HSP) is a neurodegenerative disorder, which results in progressive muscle spasticity, weakness in the lower limbs, and disrupted patient mobility. Many cases of AD-HSP are caused by mutations in SPAST (SPG4), which encodes the AAA ATPase Spastin. The Spastin protein is highly conserved across evolution, and null mutants of the Drosophila melanogaster ortholog exhibit reduced viability, physical weakness in the lower limbs, and uncoordinated locomotion. Furthermore, in the spastin5.75 null line, the larval neuromuscular junction (NMJ) had fewer microtubules, more terminal boutons, and decreased electrophysiological functioning. Exogenous expression of spastin within neurons rescued these phenotypes. The question remains whether spastin functions in other cell types, such as glia, to promote wildtype synapse formation. A forward genetic screen for spastin interactions identified the deletion of p21-activated kinase 3 (Pak3), as a suppressor of the spas5.75 mutant phenotypes. Given that Pak3 is required in subperineurial glia for this suppression, we wanted to investigate the tissue specificity of spastin function. CRISPR/Cas9 experiments were subsequently carried out with ubiquitous, glial, and neuronal promoters, generating tissue-specific deletions of the Spastin AAA ATPase catalytic domain to determine whether spastin function is required. Preliminary data suggest that ubiquitous expression had an intermediate effect, with disruptions in viability and modest increases in terminal synaptic bouton number compared to controls. However, the efficiency of CRISPR/Cas9-mediated deletion was not maximal and further optimization may demonstrate tissue-specific differences more clearly. Our class of 32 undergraduates characterized CRISPR/Cas9 animals over one semester to determine the site of mutation and the effect on animal viability and neuronal morphology. This system provided students with an excellent opportunity to learn laboratory techniques, such as animal husbandry and behavior, fine dissection and immunocytochemistry, molecular biology, and sequence analysis, all within the context of a novel research project.